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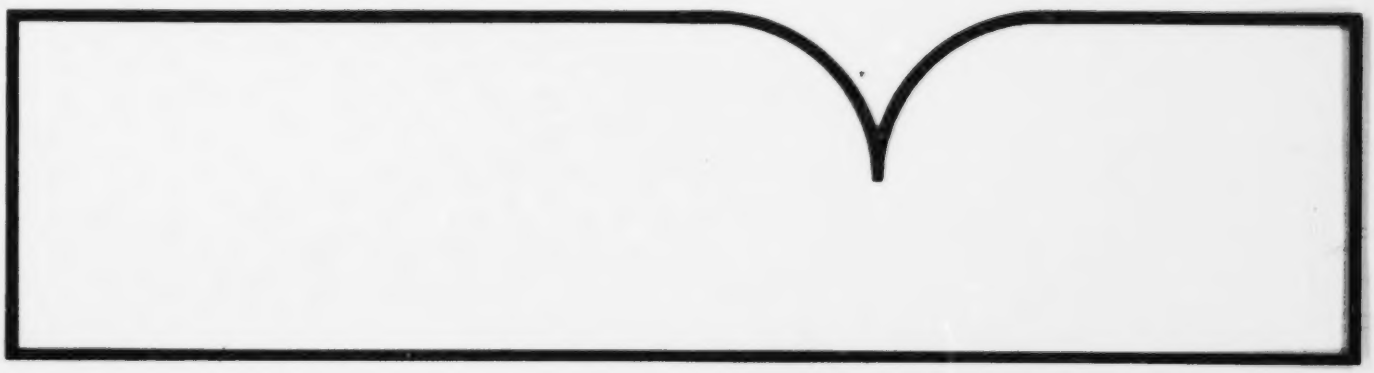
Analysis of Selected Samples for Metals Uptake

Hittman Associates, Inc.
Columbia, MD

Prepared for

Industrial Environmental Research Lab.
Cincinnati, OH

13 Mar 79



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16. ABSTRACT

Grass samples were collected from the Contrary Creek/D. Boyd Smith reclamation project of the Virginia Water Quality Control Board and the Anvil Points mine treatment experimental facility. Fish samples were collected from the EPA's mine water treatment experimental facility's sludge pond near Crown, West Virginia. Samples were analyzed for arsenic, boron, cadmium, chromium, copper fluorine, iron, mercury, lithium, manganese, molybdenum, nickel, phosphorus, lead, selenium, vanadium, and zinc. Various quality control procedures were applied and analyzed statistically in the report. The results indicated that the fish may have an unusually low tissue iron concentration and that the variable locations of pyrite tailings may be responsible for the localized spotty success of the Contrary Creek/D. Boyd Smith site. Recommendations for future research include the investigation of the low iron content in the fish as well as investigation of the spotty success of the reclamation project and the bioconcentration of elements by grasses, herbivores, and carnivores.

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April 1980

PB80-200363

DRAFT REPORT

ANALYSIS OF SELECTED SAMPLES FOR METALS UPTAKE

H-C0194/002-78-765D

by

Hittman Associates, Inc.
Columbia, Maryland 21045

March 13, 1979

REPRODUCED BY
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I. INTRODUCTION

A. Background

It is known that mining activities can result in the release of a variety of metals to the environment. For example, toxic salts of metals such as iron, copper, manganese and aluminum are often found in coal mine spoils.(1) Release of heavy metals could potentially cause significant environmental stress, for such metals can become introduced into and magnified within aquatic and terrestrial food webs. Metals uptake and/or ingestion can result in a wide range of detrimental effects on living organisms such as mortality, and metabolic process alteration. It would be beneficial, therefore, to determine the extent to which metals which are released from mining activities have been introduced into the environment. The concentration of such metals in selected floral and faunal tissues would provide valuable information relative to the extent of food web contamination. In addition, such information could aid in the development of indices for use in predicting the level of floral and faunal community impact resultant from the introduction of metals into food webs due to projected future mining activities.

In soils, the soil particles themselves influence trace elements through their ion exchange capacity, by which the trace element is adsorbed and calcium, magnesium, or alkaline earth cations are released into the soil water. Soils have a much greater exchange capacity for cations than anions.

This exchange capacity is exerted primarily by clay particles and organic colloids which tend to trap added trace elements in the surface layers of the soil. Trace metals with vacant "d" orbitals, such as copper, iron, zinc, manganese, and molybdenum tend to be bound to soil organic matter. The movement of trace elements is a function of cation exchange, water flow rate, pH, Eh, migration of soil fractions, and existing concentration of the element in the soil. Certain precipitation reactions occur at the surfaces of solid phases in the soil and they are difficult to separate from adsorption reactions. For example, cobalt and nickel may form hydrous oxides on the surface of iron hydrous oxides or clay particles. For manganese in soil an equilibrium exists between water soluble magnesium (II), exchangeable magnesium (II), colloidal hydrated mangesium oxide or mangnesium dioxide and inert mangnesium dixoide. The divalent form of manganese is soluble in acid soils, while the tetravalent form can be hydrated and reduced (either chemically or biologically) to the insoluble oxide at pH above 7.5 (2). As a consequence, for trace elements such as manganese and nickel, acidic soils make more of the element available to plants, while for selenium plant availability is facilitated by well-aerated soils with neutral or alkaline pH. As mentioned previously for selenium, plants vary greatly in their ability to take up specific trace elements.

Table 1 presents a compilation of some of the biotic and abiotic factors influencing the environmental transport of trace elements in soils. The key factors are the availability of trace elements for uptake and potential bioaccumulation by plants, ability of trace metals to travel through soil to groundwater, and immobilization of trace metals in surface layers of soil from which they can be transported to

TABLE 1. INTERACTIONS OF SELECTED INORGANIC ELEMENTS IN SOILS*

Element	Microbial Oxidation - Reduction	Bound by Soil Organic Matter	Solubilized by Acid Production or Chelation	Poor Soil Drainage Increases Availability
Zinc		+ (a)	+	
Vanadium	+			+
Thallium			+	
Tellurium	+			
Tin		+		+
Selenium	+	+		
Nickel			+	
Molybdenum	+	+		+
Mercury			+	
Manganese		+ (a)		
Magnesium	+	+ (a)		
Lead		+	+	
Iron	+		+	
Iodine	+	+		
Germanium		+		
Chromium			+	
Copper	+	+	+	+
Cobalt				
Cadmium		+	+	
Calcium		+(a)		
Bromine		+		
Aluminum			+	
Arsenic	+		+	
Sulfate	+	−		
Nitrate	+	−		
Borate		−		+
Phosphate		+	+	

(a) Relatively easy to leach from soil organic matter.

*Sources: 3,4

nearby ecosystems by surface runoff, erosion, or wind blown dust. Most non-point pollution sources can be attributed to the transport of contaminated sediments. Most cationic trace metals do not pose a threat of groundwater contamination unless they are methylated, although the anionic groups pose a potential hazard.

Chemical mechanisms act to both promote and restrict the solubility and availability of metals for uptake.

> "Other factors which affect the fate of trace elements in soils include precipitation by other ions, organic matter reactions, and soil drainage. Solubilities of cations are often limited by precipitation with phosphate, carbonates, hydroxides, or sometimes sulfates. Aluminum, beryllium, and lead are precipitated by phosphate. Barium sulfate and cadmium carbonate are both insoluble. Reactions with organic matter render some elements insoluble. Zinc humates are quite unavailable. Poorly drained or flooded soils may increase the mobility of some elements, probably because of the reduction of the elements to lower valence states in the absence of good aeration. Chromium, manganese, molybdenum, and nickel all have increased mobility under flooded or poorly drained conditions."(5)

The rate of metals uptake may be strongly influenced by temperature. Fowler and Benayoun (6) observed a 400% increase in the rate of cadmium uptake in shrimp over a 26 day period at 22°C as opposed to 8°C. Mussels were not affected by the change in temperature. Water hardness influences uptake insofar as hard water precipitates much of the cadmium. Seasonal variation in plants has also been observed (7).

Some compounds of antimony (8), arsenic (9,10,11,12) chromium (3), cobalt (3,7), copper (3,7), iron (3,7), lead (13,14,15,16,3), manganese (3,7), mercury (17,3,2), molybdenum (3), nickel (3), selenium (7), silver (7), sulfur (7), titanium (3), vanadium (3), and zinc (3), or the ele-

ments themselves are subject to microbial metabolism and undergo oxidation, reduction, methylation, and/or demethylation. The methylated forms are highly toxic, cross membrane barriers with relative ease, and are more likely to become airbone because they are more volatile (7). Microbial action also can affect the availability of many elements in soils and waters. Silicate minerals are attacked by the organic acid liberated by Bacillus siliceus (3). Organic water fermented by bacteria in the absence of oxygen can dissolve considerable amounts of cobaltous oxide, cupric oxide, ferric oxide, manganese dioxide, molybdenum trioxide, lead dioxide, and zinc and smaller amounts of chromium trioxide, nickel monoxide, titanium dioxide and vanadium pentoxide (3).

The kind and rate of microbial metabolism depends, in part, on the chemical form of the trace element. For example, alkylation of inorganic lead salts is considered a very difficult reaction mechanism in view of the instability of the postulated first intermediate, monomethyl lead salt. However, the conversion of lead nitrate and lead chloride to Me_4Pb (tetramethyl lead) by a mixed bacterial culture from Lake Ontario was reported (14). No such transformation was detected with lead hydroxide, lead cyanide, lead oxide, lead bromide, or lead palmate. The methylation of trimethyl lead acetate (Me_3PbOAc) to volatile tetramethyl lead (Me_4Pb) is, in constrast, a fairly simple transformation for bacteria to make (18).

In the laboratory, bacterial methylation activity is frequently higher under anaerobic conditions than under aerobic conditions. However, in natural fresh water the reverse seems to be true. In sediments, where anaerobic conditions are more likely to occur, hydrogen sulfide,

ubiquitous in the natural environment under anerobic conditions, combines with mercury to form insoluble mercuric sulfide. Thus, under aerobic conditions in fresh water, methylation rates are likely to be higher than under anaerobic conditions, where the formation of mercuric sulfide binds mercury and hinders methylation (19).

B. Bioconcentration

Bioconcentration refers to the ability of an organism to accumulate a pollutant above the ambient level. This phenomenon may result from an energy-requiring active transport mechanism by which the organism concentrates a micronutrient from the environment. However, the phenomenon also occurs if the pollutant is sequestered in the organism. This sequesteration may result from chemical reaction (for example with protein sulfhydryl groups) or physical phenomena (for example, a lipophylic compound dissolving in high lipid tissue and thus removing itself from the excretion process). Higher aquatic organisms such as fish can incorporate arsenical compounds and metabolize them yielding high-molecular weight arsenic-containing lipid material (9).

1. In Plants

When soil concentrations of essential micronutrients such as manganese, zinc, cobalt, copper, and molybdenum are increased, plants continue to accumulate them--sometimes to toxic levels. Nonessential elements such as cadmium or nickel may also be bioaccumulated. The uptake of nonessential elements may be affected in part by the fertility of

the soil and ultimately, by the general nutrition of the plant. In a series of experiments, corn plants grown with insufficient levels of phosphate in sand cultures accumulated five to ten times more added lead (as lead nitrate) than plants provided with sufficient phosphate levels. Phosphate-sufficient plants also were less sensitive to the lead that was accumulated. Lead was added in quantities high enough to insure that decreased accumulation was not just the effect of lead phosphate precipitation (5).

Other conditions such as plant growth period, age, or water stress may affect uptake of trace elements, and plant rooting characteristics are also likely to be important. For example, most the the acetic acid-soluble lead, cadmium, and zinc was found in the upper 3 cm of heavily polluted soils near a large lead and zinc smelting complex. In these soils, plants with shallow, spreading roots are probably exposed to more toxic concentrations than deeper rooting species. Also, plants with greater root surface area generally have more potential for uptake (5).

Unfortunately, the bioconcentration ratio (concentration in the plant/concentration on the water) even for a single plant species is not constant but is a function of the available (total, extractable or soluble soil fraction) pollutant level in the soil. The bioconcentration level generally increases with decreasing soil concentration. The bioconcentration depends on the uptake (minus the amount lost) and, hence, is subjected to all the variables discussed previously. In addition, uptake rate appears to reach a maximum in those experiments with sufficient data to plot saturation kinetics.

2. In Animals

The bioconcentration factors for animals are also functions of the difference between uptake and elimination and are subjected to all the factors affecting uptake as discussed previously. Weir (7), using the data of Mason and coworkers (20) on mercury uptake by the oyster, Hermione hystrix, calculated the following relationship between the bioconcentration factor (y) (which ranged from 441 to 1230) and the water concentration which ranged from 10 to 100 parts per thousand (ppt):

$$y = 1300-9.4x \qquad (r = 0.982,\ p < 0.02)$$

where: y = the bioconcentration factor (concentration in the fish/concentration in the water)

x = concentration in the water (ppb)

r = Pearson's product moment correlation

p = probability that the observed relationship is due solely to chance.

Again, Weir (7) used data of Mason and coworkers (20) on the bioconcentration factors of fathead minnows, Pimephales promelas, exposed to methylmercury concentrations ranging from 0.015 to 0.240 parts per billion (ppb). The bioconcentration factors ranged from 45 to 98 and conformed with the following formula:

$$y = 19.4-(18.7)(\ln x) \qquad (r = 0.997,\ p < 0.001)$$

These two equations demonstrate that bioconcentration factors are not constant with differing water concentrations but are related to the water concentration in realtively complex ways (20).

Table 2 gives ranges of bioconcentrations found for several classes of organisms. These ranges are broad because different species are considered and because the bioconcentration factors are functions of several variables as discussed previously. In addition to the variables discussed previously, bioconcentration factors are functions of inorganic and organic water chemistry and food web dynamics (21). Bioconcentration is a complex process dependent to a great extent on the organism in addition to other factors such as physiochemical parameters such as competing ions, ligands, complexes, colloidal suspendsions, pH, temperature, etc. Before the use of bioconcentration factors can be relied upon, careful consideration must be given to the species involved, its growth and nutrient requirements, feeding habits, and metabolism in various environments.

In addition to the information contained in Table 2, the bioconcentration factors for earthworms are 17.4-22.5 for cadmium, 0.2-53 for lead, 1.2-2.6 for nickel, and 7.7 for zinc. The bioconcentration factor for terrestrial vertebrates is from 0.3 to 18.7 for cesium, 0.40 to 8.4 for strontium, 0.2 to 2.4 for cobalt, and 0.1 to 0.5 for iodine (2).

The following elements showed possible discrimination against food chain transfer and bioconcentrations in that sediments contained higher content than detrital eaters, or detrital eaters and herbivores contained higher lead concentrations than did carnivores: lead (21, 32, 36), selenium

TABLE 2. BIOCONCENTRATION FACTORS FOR VARIOUS ELEMENTS IN VARIOUS GROUPS OF ORGANISMS*

Element	Microorganisms Including Single-celled Algae, fungi, bacteria	Aquatic Plants (Including Marine Plants) Including the Kale, Brassica oleraced	Non-Aquatic Non-Agronomic Plants	Agronomic Plants	Marine Invertebrates	Freshwater Invertebrates	Marine Vertebrates	Freshwater Vertebrates
Aluminum	1550-25000		0.01					
Antimony	50	27-127		0.13-1.46	5	10	40	1
Arsenic	200-71000	50-71000	0.03	0.37-1.23	9-64,100	80-480	38-12,150	1-333
Barium	108-260		0.11					
Beryllium	1000-1500		0.03	2-16	200	10	200	2
Bismuth							15	15
Boron	6.6							
Bromine	2.8				1		1	
Cadmium	12500	1000-1620	5.3	0.21-1.47	10-250,000	200-30600	3000	30-2000
Calcium	0.5-10	13-265						
Cerium		7100	0.5					
Cesium	1-100	480	0.03					
Chlorine	0.062-1.0	38						
Chromium	0.5-17000	695-10,000	0.000-0.082	0.01-250	0.1-2000	20-3000	200-400	20-200
Cobalt	277-4600	4425	0.0-0.1	0.019-87	1000	200	100	20
Copper	100-17000	275	1000	0.02-90	50-10,000	1000	667	200
Fluorine	0.86				1		1	
Gallium	4200-12000		0.04					
Germanium	15-200		0.25		15,700	33	3300	3300
Iodine	1200-10000	370						
Iron	2000-140,000	120-4935						
Lathanium	8300							
Lead	360-70,000		0.04-2.3	0.44-12	1000-3500	100	300	300
Lithium	8		1					
Magnesium	0.59-0.96	17			1			
Manganese	750-9400	100-100,000	3000	0.41-3	100-10,000	40,000	600	100

*Sources: 3-6,8,13,22-29,30-35

TABLE 2. BIOCONCENTRATION FACTORS FOR VARIOUS ELEMENTS IN VARIOUS GROUPS OF ORGANISMS (Continued)*

Element	Microorganisms Including Single-celled Algae, Fungi, Bacteria	Aquatic Plants (Including Marine Plants) Including the Kale, Brassica oleraced	Non-Aquatic Non-Agronomic Plants	Agronomic Plants	Marine Invertebrates	Freshwater Invertebrates	Marine Vertebrates	Freshwater Vertebrates
Mercury	196-42000	12-5915	0.05-42.8	0.009-26.2	1-33,300	100-100,000	1670	45-70,000
Molybdenum	11-1700		0.42-900	55-582	10	10	10	10
Nickel	140-1700	260-1700	0.014-1500	0.008-331	0.08-1000	100	100	100
Niobium	450-1000	7640	0.11					
Phosphorus	10,000-15,000	1600-5480						
Potassium	3-50	115						
Radium	100-4500			84.6-1787	100	250	50	50
Rubidium	5-50	1230	0.02					
Scandium	1500-2600		0.001					
Selenium	2290-7230	0.5-2000	1-1000	0.05-10	1000	167	4000	30-167
Silicon	120-17000							
Silver	6-250,000				1-200,000	0.7-1,400		0.7->700
Sodium	0.14-1.0	19					1	
Strontium	1.0-44	475	0.09					
Tellurium				0.64-4.60	100,000	75		400
Thallium	50				10.9-15,000	15,000	10,000	10,000
Thorium	10		0.004-0.18		2000	500	10,000	30
Tin	92-2900		0.09	0.0003-0.25	1000	1000	3,000	3,000
Titanium	3000-20,000		0.001					
Tungsten	87			2.3-24	30	10	30	1200
Uranium	10							
Vanadium	250-1900		0.008	0.011-2.65	50	3000	10	10
Yttrium	100-1000	6680						
Zinc	100-208,000	41-2830	40	0.31-39.0	549-260,000	125-16,400	1050-35,400	39-2400
Zirconium	350-3000	6230	0.03-1.56	1.4				

*Sources: 3-6,8,13,22-29,30-35

(21), copper (37), chromium (38), arsenic (7), cadmium (39,21,40), and nickel (41,42,43).

Bioconcentration is an important concept because toxic concentrations can be accumulated from non-toxic environmental concentrations. This concentrating effect may be sufficient to cause food concentrations from barely detectable levels to levels which cause chronic manifestation of symptoms years after exposure.

C. Purpose and Objectives of the Study

In addressing this metals uptake question, the U.S. Environmental Protection Agency contracted Hittman Associates, Inc. (HAI) to obtain and analyze floral and faunal samples from three mining related sites. The objectives of this study were the following:

(1) To obtain selected floral or faunal samples from three mining related sites at the direction of EPA/Cincinnati.

(2) To analyze the samples obtained in order to determine the concentration of various parameters specified by EPA in the floral/faunal tissues.

(3) To develop a final report describing the sampling program conducted, the samples obtained, the methodology used in analyzing the samples, and the results of the sample analyses.

D. Methodology

The approach utilized in achieving the above-cited objectives initially involved visiting the following three sites, at the direction of EPA/Cincinnati, to obtain the types of samples indicated:

(1) The EPA Crown Coal Mine Wastewater Treatment Facility, located near Morgantown, West Virginia. Two samples of fish living in the on-site sludge lagoon were collected.

(2) The site of EPA Project 803801, "Control of Metals in Contrary Creek" located near Charlottesville, Virginia. Ten samples of vegetation growing thereon were obtained.

(3) The EPA Project R 804719 site, "Vegetative Stabilization of Spent Oil Shale", located near Grand Junction, Colorado. Seven samples of vegetation from spent shale reclamation areas were collected.

Sample collection and handling were conducted so as to assure that the samples would not be contaminated prior to analysis. Sample preparation and analysis were carried out at the Hittman Associates laboratory facility in Columbia, Maryland. All procedures were conducted in accordance with applicable EPA Protocol II for laboratory analyses. The following sections provide detailed information concerning the sites visited, the sampling methods utilized, the types of samples obtained, the laboratory procedures used in preparing and analyzing the samples, and the results of the analyses.

II. FIELD SAMPLING PROGRAM

A. Crown Coal Mine Wastewater Treatment Facility

1. Site Description

On November 6 and 7, 1978 Hittman Associates field team visited this coal mine wastewater treatment pilot facility established by the U.S. Environmental Protection Agency (EPA) near Morgantown, West Virginia (Figure 1). Sludge from mine waters treated at the facility is pumped into an on-site settling pond. Fish, turtles, as well as ducks and other birds, have been sighted in the pond. Deer are also known to frequent the site (44).

The EPA has previously obtained and analyzed samples of the pond water and pond fauna, sludge samples, and samples of raw feedwater into the treatment plant. Table 3 presents illustrative results of such analyses. As the data in the table indicate, except for calcium, high levels of metal contaminants have been found in the raw feedwater as compared to pond water. However, concentrations are in many cases noticeably reduced in the pond waters themselves. While past analyses by EPA have identified calcium, sodium, magnesium, iron, manganese, potassium, aluminum and small amounts of zinc and copper as being present in the pond, the ultimate fate of these contaminants has not been conclusively determined.

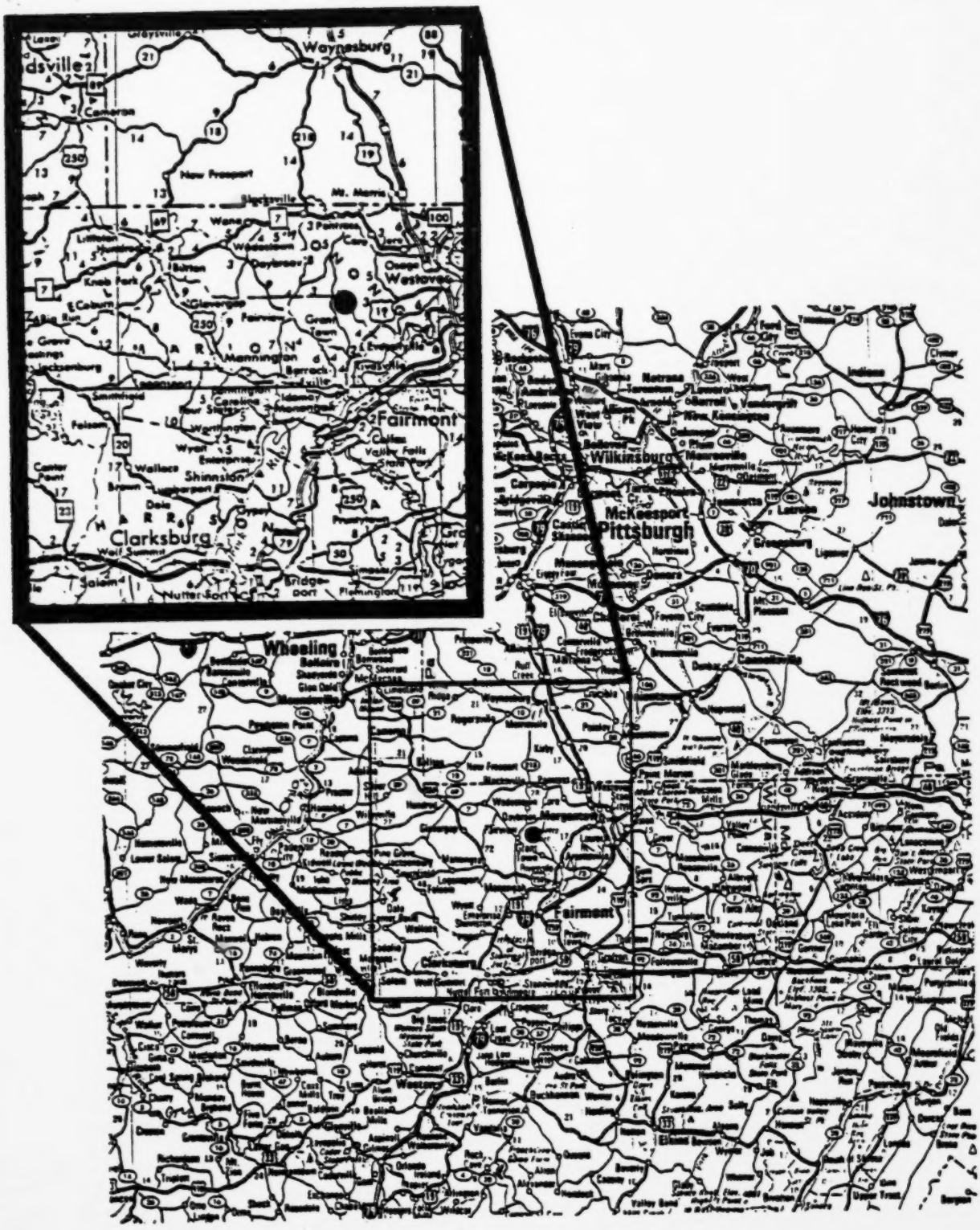


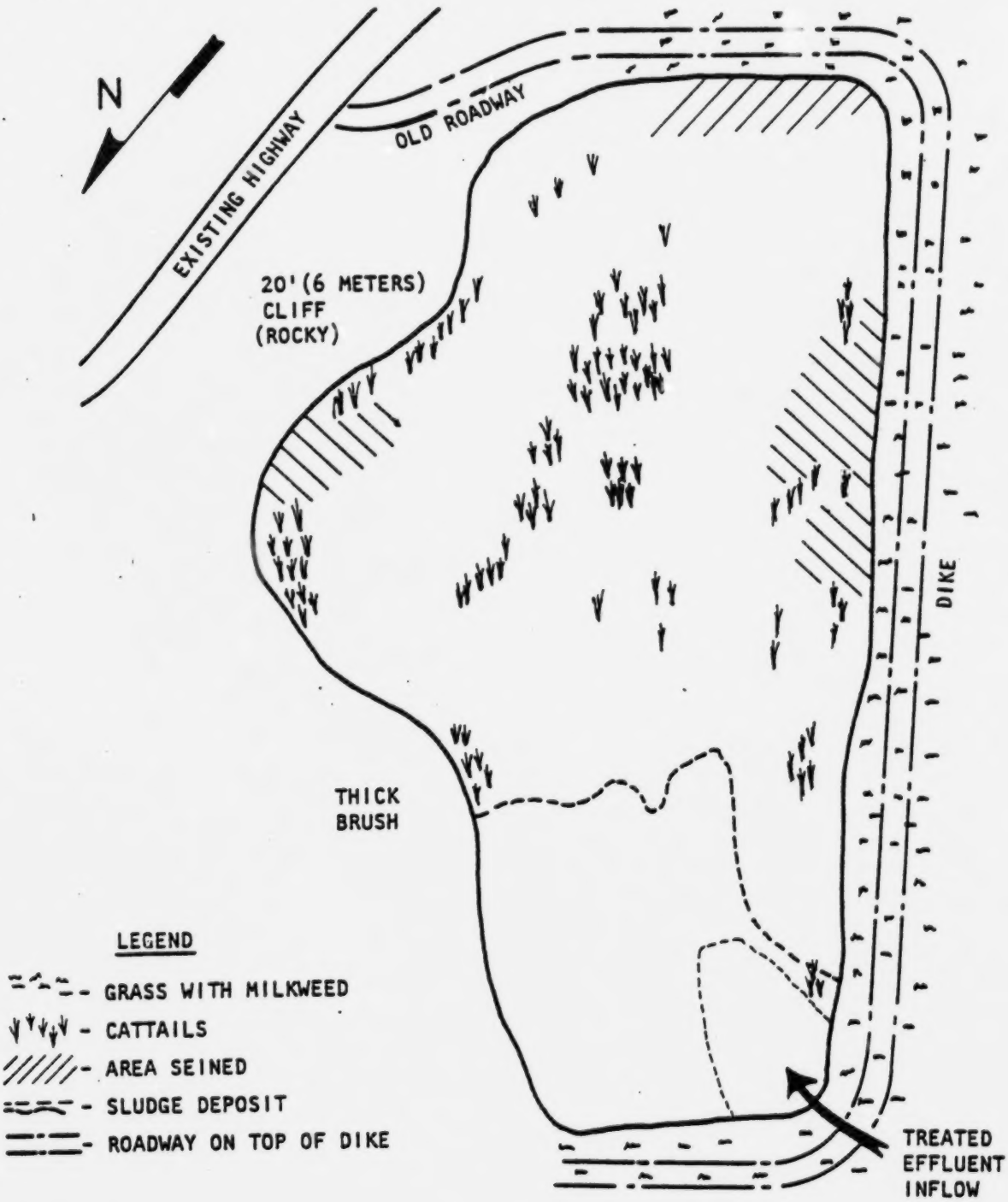
Figure 1. Crown site (West Virginia) location map

TABLE 3. WATER ANALYSIS: CROWN SITE RAW FEED AND SETTLING POND (44)

Raw Feedwater (October 6, 1978)			Settling Pond Water (April-May 1978)[4]		
pH	5.3		pH	6.5	
Cond	3330	(micro-mhos/cm.)	Cond	2200	(micro-mhos/cm.)
Acid	922	($CaCO_3$ eq.)	Acid	6	($CaCO_3$ eq.)
Ca	440	(mg/1)	Ca	460	(mg/1)
Mg	140	(mg/1)	Mg	42	(mg/1)
Fe^{+3}	220	(mg/1)	Fe^{+3}	.42	(mg/1)
Na	460	(mg/1)	Na	270	(mg/1)
Al	5.0	(mg/1)	Al	.08	(mg/1)
Mn	4.8	(mg/1)	Mn	.29	(mg/1)
Alk	22	($CaCO_3$ eq.)	Alk	20	($CaCO_3$ eq.)
Turb	15	(J.T.U.'s)	Turb	8.8	(J.T.U.'s)
TSS	21	(mg/1)	TSS	316	(mg/1)

The HAI field team obtained samples of the fish living in the on-site sludge pond under the direction of the EPA representative in charge of the Crown facility. The sludge pond was quite turbid at the time of the visit. In general the waters exhibited a greenish brown color, with the exception of the water immediately in the area of the treated water inflow point, where a slight orange tint deriving from the sludge was observed. This turbid condition had been observed for a period of eight days prior to the arrival of the HAI field team (44).

This turbidity occurred after the addition of an experimental phosphate treatment step in the overall mine water treatment process stream. Specifically, mine wastewaters were allowed to flow through a tank containing phosphate in order to increase the phosphate content of the sludge produced thereby increasing its potential commercial value as a soil fertilizer. Prior to the phosphate treatment the pond had been clear, and it was possible to see the bottom and observe fish therein.(44) Figure 2 presents an approximate sketch of the pond. Aside from some deep holes



NOTE: Dike is approximately 6' (2 METERS) above surface of water.

Figure 2. Crown sludge lagoon

(8 to 10 feet (2.4 to 3.1 m) in depth), the average depth is approximately four feet (1.2 m), dropping considerably from the middle to the southern end. At the intake the water is roughly one foot (0.3 m) in depth. Here the sludge was visible and it extended for a distance of roughly twenty yards (18 m) from the shore. Generally, the bottom substrate was found to be composed of soft muck. There was no observed outlet from this pond into other waterbodies. Cattails (Typha sp.) were noted growing in patches at various locations within the pond, and the shoreline is primarily vegetated with milkweed (Asclepias sp.) and various grasses.

2. Sample Collection and Handling

For this study, two smallmouth bass (Micropterus dolomieu dolomieu Lacepede) approximately nine inches in length and approximately three years in age* were obtained by use of a 30' x 4' (9.1 m x 1.2 m) seine net and waders. Due to the presence of cattail clumps at various intervals along the pond's shoreline and the depth of water in other areas, that portion of the pond wherein seining could be conducted was limited. Locations at which the fish samples were obtained are indicated on Figure 2.

In order to avoid sample contamination, care was taken to assure that the fish obtained did not come into contact with metallic substances. Following seining each fish obtained was placed in a separately labeled Zip-lock bag and stored in an insulated plastic cooler filled with dry ice so as to preclude decay of body tissues during transport to the HAI laboratory.

Age determined by fish scale examination.

B. Contrary Creek Reclamation Project

1. Site Description

The Contrary Creek, Virginia study area actually consist of two sites, namely the "Sulfur Mine Site" and the "D. Boyd Smith Site". Figures 3, 4 and 5 present location maps for these two sites. The sites encompass old sulfur mines and sulfur mine tailings piles. Both sites are presently barren except for the grasses which have been successfully nurtured under the site reclamation project. The sites are surrounded by Virginia pines (Pinus virginiana) and eastern white pines (Pinus strobus). There is little hardwood succession at the Sulfur Mine Site, while yellow poplar (Liriodendron tulipifera), post oak (Quercus stellata) and red maple (Acer rubrum) are invading the pines surrounding the D. Boyd Smith Site.

Both sites are drained by creeks. The Sulfur Mine Site is drained by Contrary Creek and the D. Boyd Smith Site is drained by a tributary to Contrary Creek. The creek water at both sites was clear. The bottoms of both creeks are red which may indicate high iron content in the water. Generally no plants grow on the banks of or in the creeks. Some white grass (Leersia virginica Willd.) which had died off due to the oncomming winter was reported in the creeks.(45)

The only signs of wild mammals observed at the two sites were deer tracks. However, the relative scarcity of such tracks indicate that these deer infrequently visit the site. No rabbit or squirrel sign was found during the period of sampling.

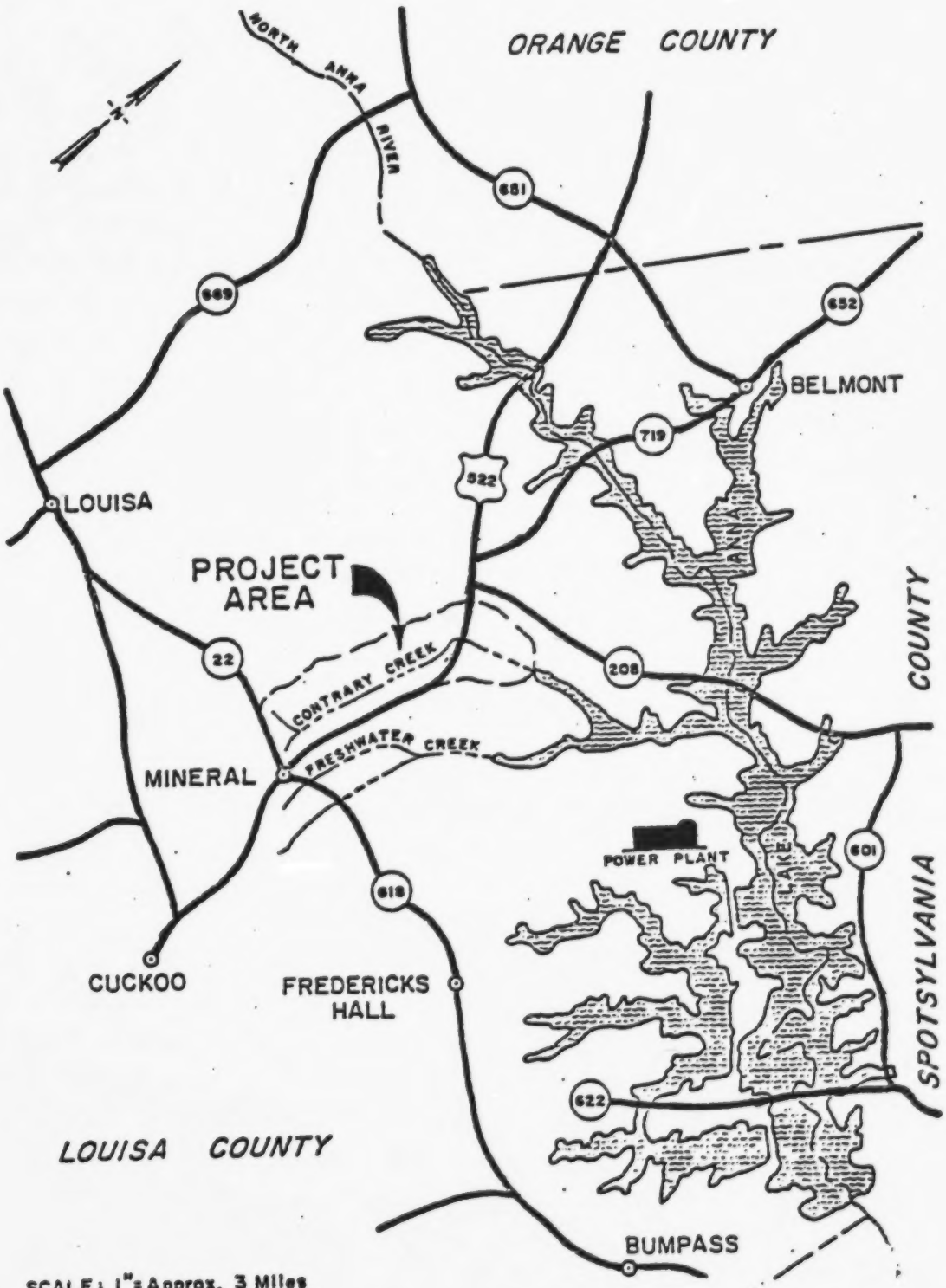


Figure 3. Location of Contrary Creek project area

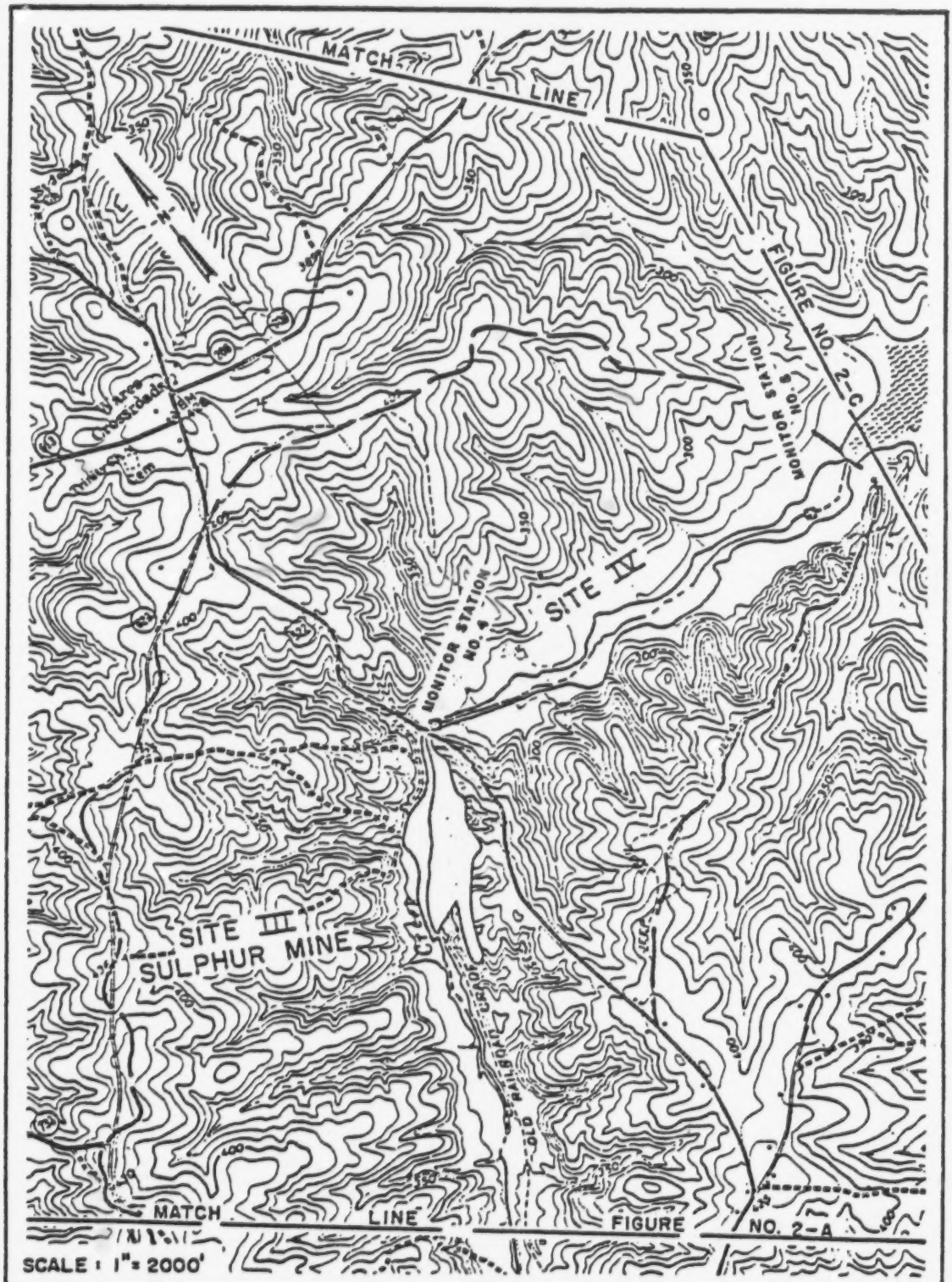


Figure 4. "Sulfur Mine" reclamation site

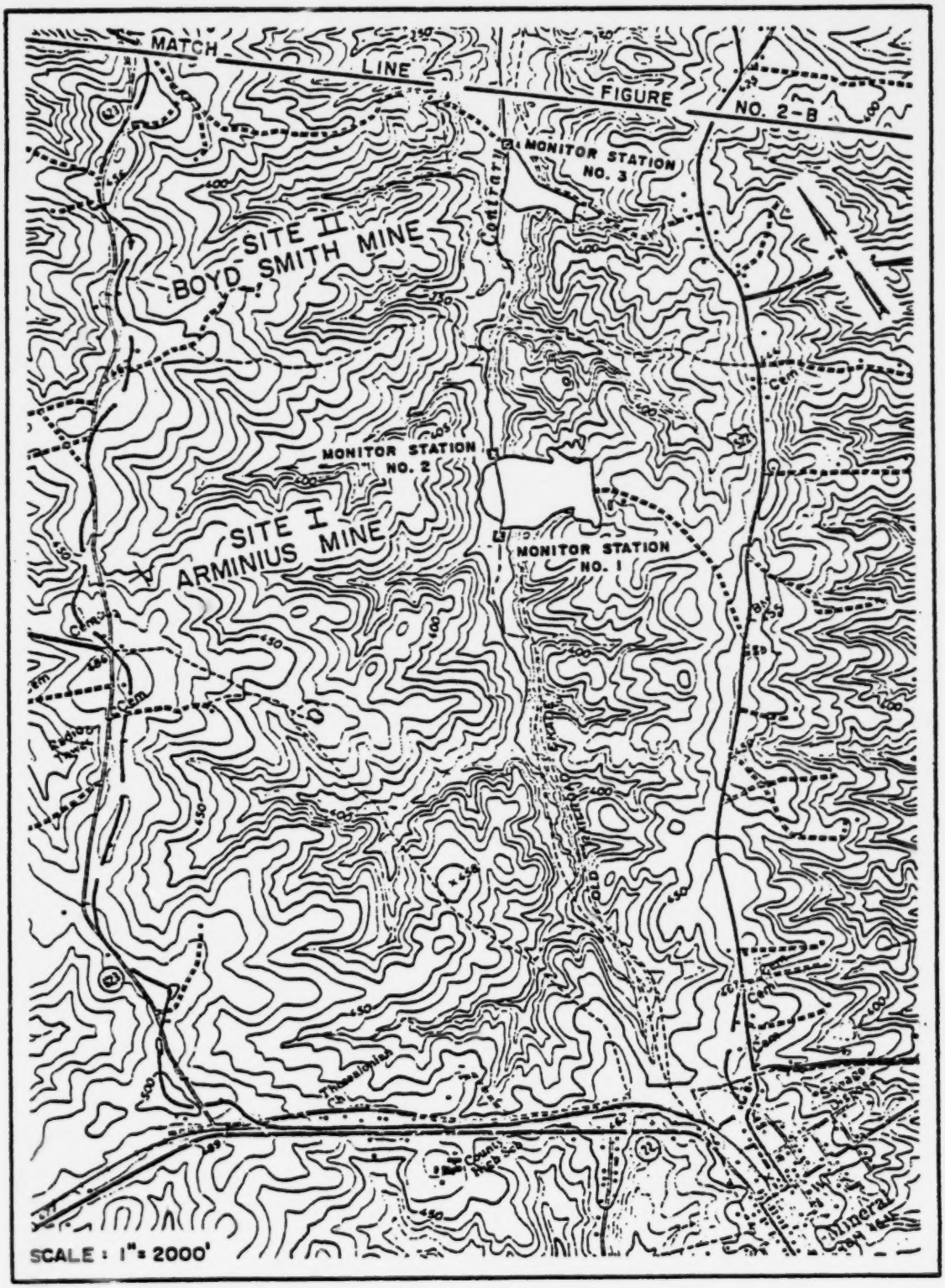


Figure 5. "D. Boyd Smith" reclamation site

Surface sulfur mines were active here after 1830, although only deep shaft mining was conducted between the 1880's and the 1920's.(45) For the following 50 years the sulfur mines were inactive; during this time little vegetation grew and survived. In the 1960's a paper company in the area tried planting pine seedlings, although only a few of these survived. When the site reclamation project started in 1976 the area was vegetated with only scattered Virginia pines and some grasses.

Pyrite (FeS_2) was the principal form of sulfur mined. Geological information indicates that the tailings should also contain lead, gold, silver, copper, manganese and zinc. The only on-site processing was grinding and sizing, and no ore refining occurred at the site. Since a railroad carried the ore away, mine tailings (characterized by acidity and heavy metals content) constituted the only source of environmental pollution.

During reclamation of the site approximately 10,000 tons (9100 Mg) of Washington, D.C. sewage sludge was spread over the entire area. Some locations received two to five applications depending on the plant growth rate. The exact amount of sludge applied to each site is difficult to quantify, but each has received at least one sludge application. The area has been seeded five times in all with grasses such as weeping love grass (Eragrostis sp.), fescue (Festuca sp.), and rye (Secale sp.). The rye was applied as a nurse crop, and the love grass was seeded only in the spring. Only areas in which the grasses did not take were reseeded. The seeds were applied in 1976, the spring of 1977, the fall of 1977, the spring of 1978, and the fall of 1978. Fertilizer has also been added with the last addition being made in the spring of 1977. All recently reseeded

areas received lime as well as sludge in late September, 1978. In addition to grasses, the paper company tried planting 17,000 pine seedlings last year, but essentially all of them died.(45)

Figure 6 (following page) presents an illustration of the specific EPA-designated sampling stations at the Sulfur Mine site. Station 1 is a control site located above the mine site and vegetated with a variety of escaped grasses. Station 2 is presently supporting growths of clover, rye and fescue. It has not been recently reseeded. Stations 3 and 4 and parts of stations 7 and 8 and the sludge storage areas are the only recently reseeded sites (Fall 1978). These sites were reseeded with fescue and rye. Stations 7 and 8 were relimed in the fall. Station 8 has been reported to be a major problem area in terms of acidity. It was reseeded in the fall of 1978 with fescue and lovegrass. Station 2 is known as the "sandwich area". This term is applied because layers of sludge and lime have been applied one on top of the other as is indicated in Figure 7. Vegetative cover is doing quite well in this area.

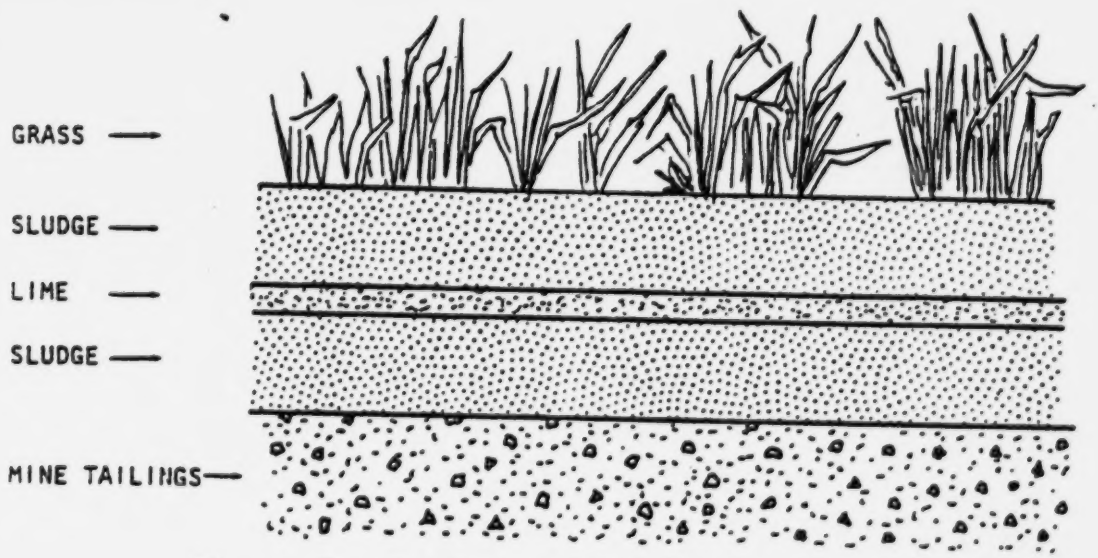


Figure 7. Profile of "sandwich area"

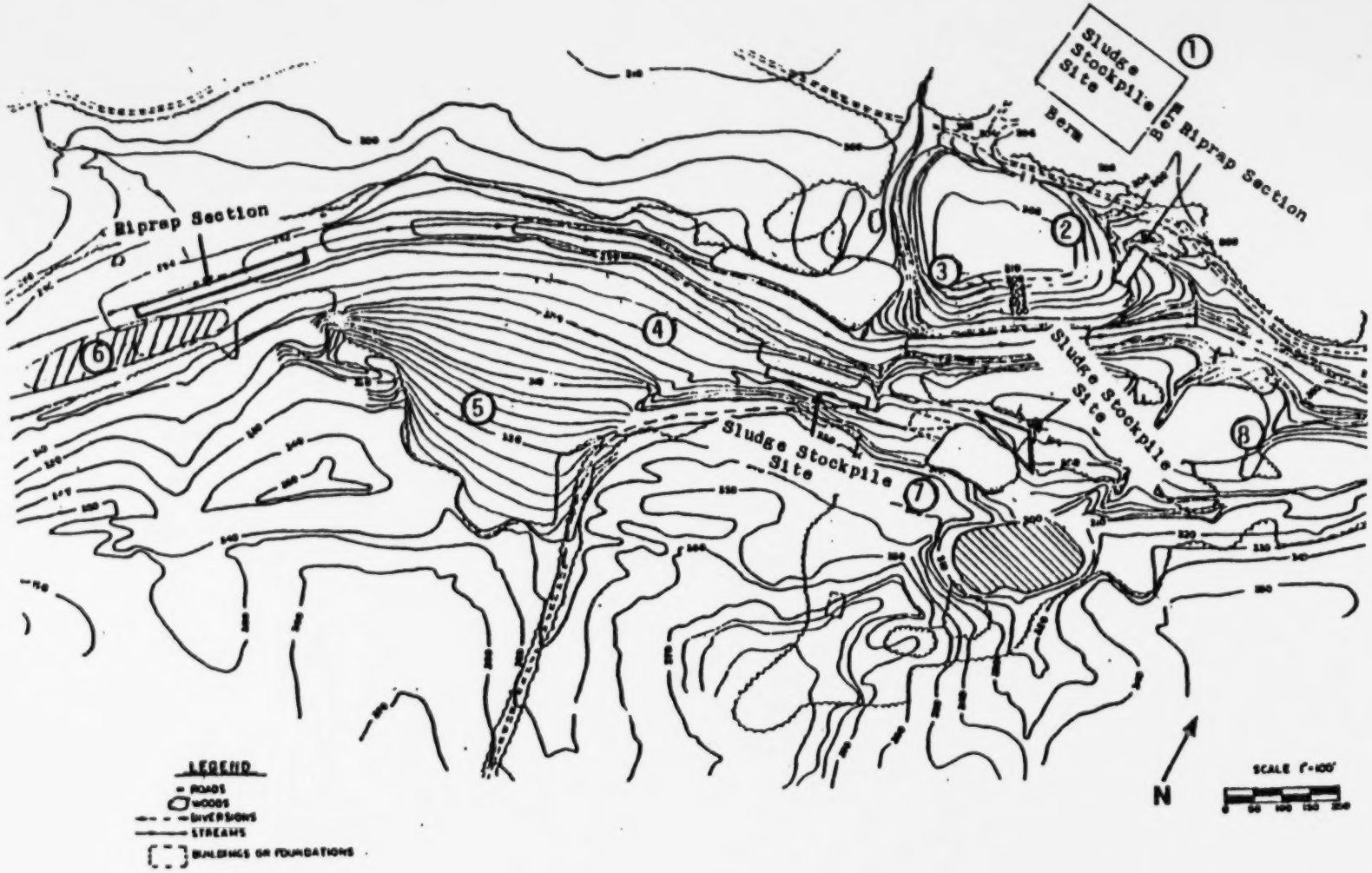


Figure 6. Sulfur mine sampling locations (circled)

Figure 8 illustrates the specific sampling stations where grass samples were obtained from the D. Boyd Smith site.

During the 1977 growing season the study area received record setting low rainfall which proved a significant deterrant to reclamation efforts. In order to avoid this problem should it recur in the future, irrigation systems are planned for use during future growing seasons to supplement natural precipitation. Finally, it is interesting to note that several types of fruits and vegetables have spontaneously sprouted at the site. Specifically tomatoes, watermelons, cantaloupes, cucumbers, and squash have been noticed. It is assumed that the seeds from which these plants grew were transported to the site with the sludge.

There are five monitoring stations along Contrary Creek which provide water quality analyses and flow measurements. Approximately four soil and imported sludge analyses have been made since the reclamation program started.

2. Sample Collection and Handling

Grass samples were obtained from the sulfur mine and D. Boyd Smith sites on November 9, 1978. Table 4 provides an approximate determination of the species mix as well as the total percent cover at each sampling station. As a number of grass species were present to varying degrees at each sampling station, composite samples were obtained. These samples roughly approximated the species mix at each station. Samples were obtained by hand and care was taken to insure that no metallic contamination of the samples occurred. Only the above-ground portions of the plants (no roots) were taken in order to obtain samples of those portions of plants

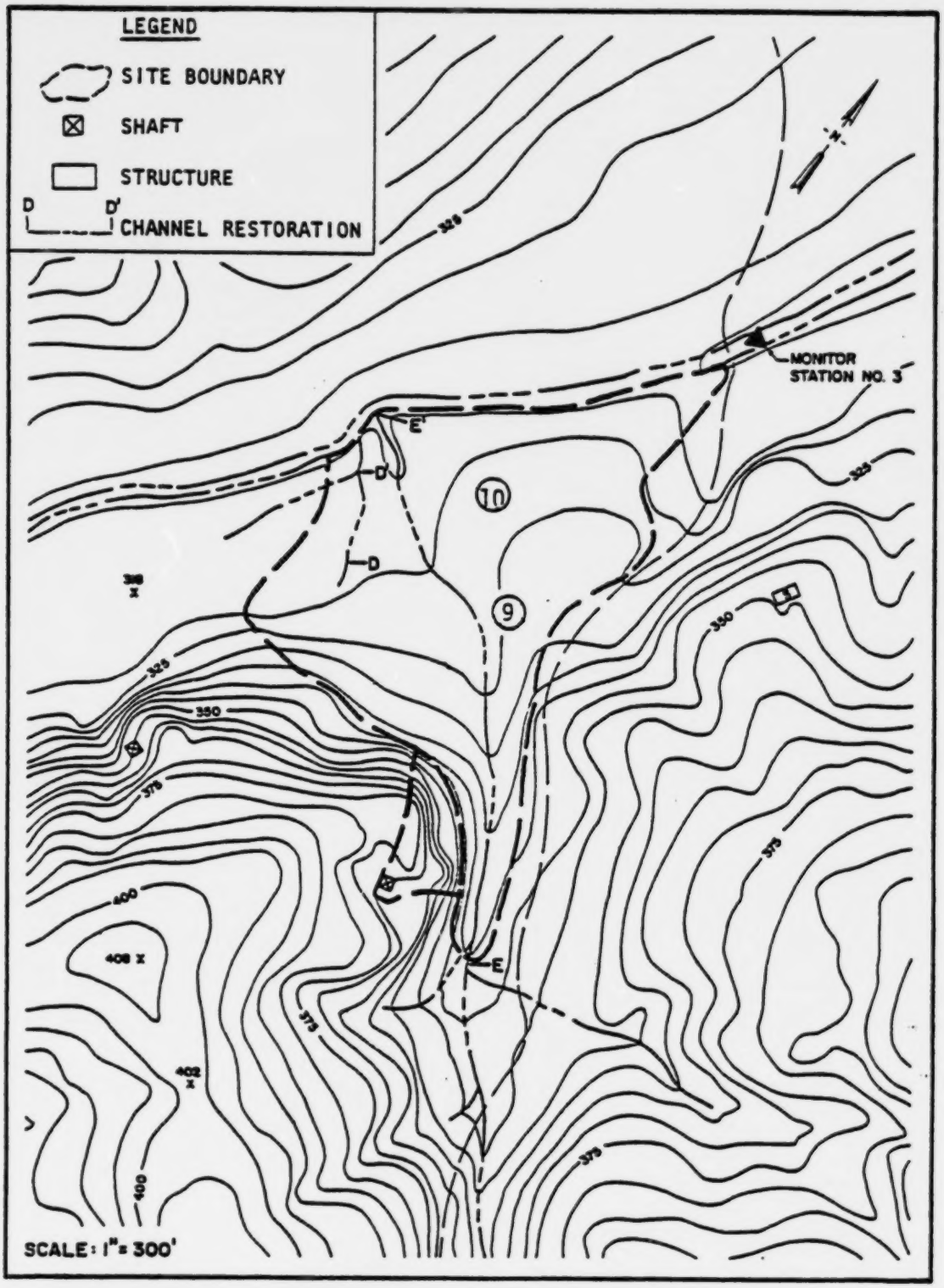


Figure 8. D. Boyd Smith sampling locations (circled)

TABLE 4. SAMPLING LOCATION SPECIES MIX AND PERCENT COVER

Sample No.	Species mix/% cover
o Sulfur Mine Site:	
1	Control - escaped grasses: 100% cover
2	Clover (3%), Rye (47%), Fescue (50%): 90% cover
3	Rye (66%), Fescue (33%), Clover (1%): 10% cover
4	Rye (50%), Fescue (50%): 10% cover
5	Lovegrass (20%), Fescue (80%): 90% cover
6	Lovegrass (50%), Rye (20%), Fescue (30%): 50% cover
7	Lovegrass(15%), Fescue (85%): 95% cover
8	Rye (70%), Fescue (30%): 5-15% cover
o D. Boyd Smith Site:	
9	Fescue (85%), Lovegrass (15%): 5% cover
10	Fescue (85%), Lovegrass (15%): 90% cover

to which grazing animals would be exposed. Also, this approach minimizes the potential for sample contamination due to adhering soil particles. Each sample was placed in a plastic Zip-lock bag and labeled for identification. The samples were all transported to the HAI laboratory facility in Columbia, Maryland in a plastic cooler filled with dry ice.

C. Anvil Points Reclamation Project

1. Site Description

This reclamation project is located near the Paraho oil shale processing site at Anvil Points, about ten miles (16 kilometers) west of Rifle, Colorado (see Figure 9). At the site, reclamation plots have been established to test the

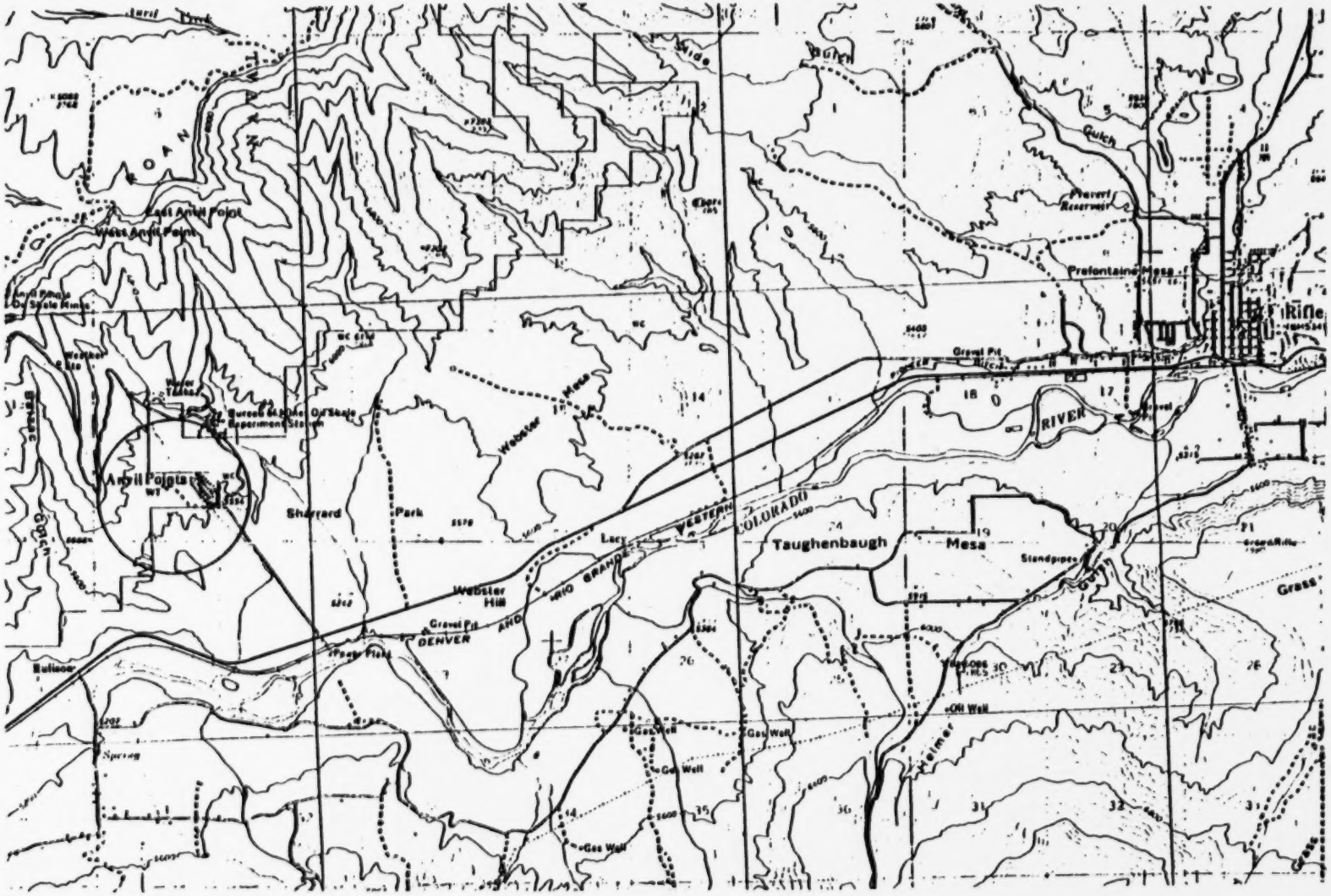


Figure 9. Anvil Points reclamation site: location map

reclamation potential of three types of oil shale tailings. The tailings under evaluation are those from the Tosco process, the Paraho process, and from the U.S. Bureau of Mines (U.S.B.M.) mining which occurred in the 1940's. Reclamation plots have been established at two sites, located about one-half mile apart and two miles southeast of the processing plant (see Figure 10). At the northern site, which is the older of the two, reclamation success is being evaluated using tailings from the 1940's U.S.B.M. oil shale mining. Also, more recent tailings from the Tosco process have been transported into this site for reclamation evaluations. Paraho tailings are present at the southern site. Each reclamation plot is 22 feet (6.7 m) square and is equally divided along the north/south central axis. Also, the plots are fenced-in so that wildlife can not graze the revegetated areas. Reclamation is being evaluated at the sites on bare tailings alone as well as on tailings covered to some degree with topsoil. Samples were obtained from both the bare and covered plots.

2. Sample Collection and Handling

Sampling was conducted on November 1, 1978. The Environmental Protection Agency (Cincinnati) designated the reclamation plots to be sampled, and also indicated that the fourwing salt grass (Atriplex canescens) was to be the target species in this sampling effort.(46) Samples were acquired by hand using no metallic instruments so as to preclude the potential for sample contamination. The samples were taken from the following reclamation plots:

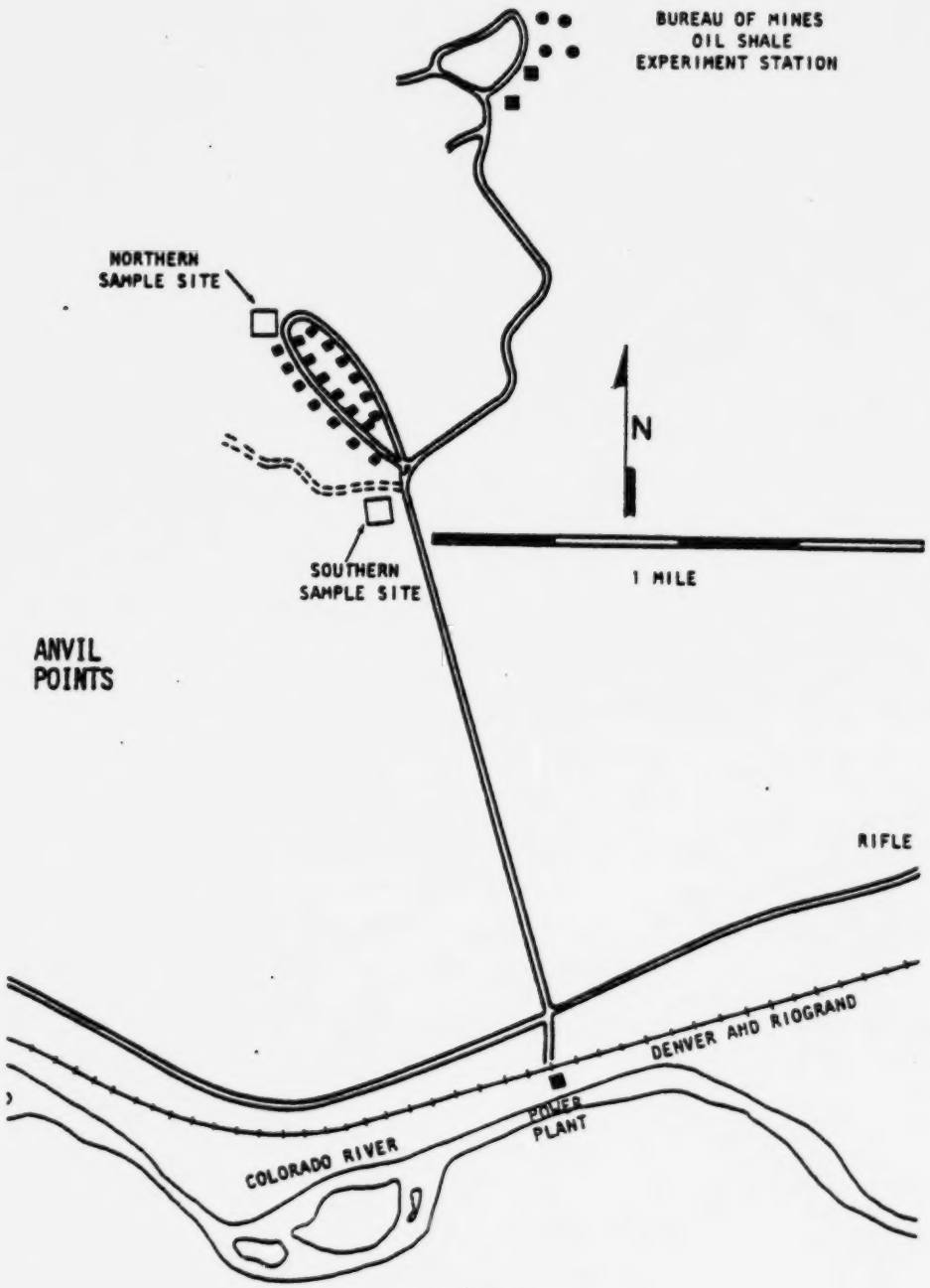


Figure 10. Anvil Points reclamation site: detail map

Sample #11 — Tosco - tailings alone (approximately 60% vegetative cover)

Sample #12 — Tosco - tailings covered with 30 cm topsoil (approximately 95% vegetative cover)

Sample #13 — USBM - tailings alone (approximately 70% vegetative cover)

Sample #14 — USBM - tailings covered with 15 cm topsoil (approximately 95% vegetative cover)

Sample #15 — Paraho - tailings alone (approximately 15% vegetative cover)

Sample #16 — Paraho - tailings covered with 40 cm topsoil (approximately 50% vegetative cover)

Sample #17 — Control - mix of vegetation from the USBM bare tailings plot (approximately 70% vegetative cover)

Samples analyzed for metals content were those taken from the east-facing half of each reclamation plot.

Vegetation growing on the plots varied. Some plots supported only the four wing salt grass. Other plots supported this species as well as others such as winter fat (Eurotia lanata), western wheatgrass (Agropyron Smithii) and a summer grass. However, as has been previously mentioned only samples of the fourwing saltgrass, which was present on all plots, were analyzed for metals content. Only the above-ground portions of plants (no roots) were taken in

order to obtain samples of those portions of plants to which grazing animals would be exposed. Also, this approach minimizes the potential for sample contamination due to adhering soil particles. Samples obtained were composites in that portions of a number of individual plants within the sample plot area were obtained in order to make up a single sample. After each sample was obtained, it was handled in the same manner as has been described for the Contrary Creek samples. However, the Anvil Points samples were obtained after the end of the western growing season and were quite dessicated. It was determined, therefore, that they required no refrigeration in order to preserve the integrity of plant tissues during shipment.

III. LABORATORY ANALYSIS*

A. Preparation of Grass Samples

All grass samples were washed with distilled, deionized water and placed in acid-washed beakers of appropriate size (1-2 liters depending on sample size). The beakers were placed in a drying oven and maintained at 80°C for 15-18 hours until visually dry. After cooling, the beakers were covered with parafilm and stored until the samples could be homogenized and analyzed.

A large mortar and pestle was used to hand-grind the samples to the best possible mixture. A portion of each sample (from 1-10 grams depending on the amount of sample) was divided into two acid-washed beakers and was weighed.

One of these portions was subjected to dry ashing (for all elements analyzed except arsenic, phosphorous, mercury, and selenium) and the other portion was subjected to wet ashing (for arsenic, phosphorus, mercury and selenium analyses). An additional portion was analyzed for boron and fluoride after treatment with saturated calcium hydroxide followed by ashing.

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*References 47, 48, 49, 50, and 51.

1. Dry Ashing

The portion to be dry ashed was placed in a muffle furnace at 500°C for 3 hours. The ash was cooled and dissolved in 5 ml of 20% v/v hydrochloric acid. If the ash did not dissolve completely the suspension was heated to dissolve the residue. The solution was then filtered through an acid-washed filter paper (0.45 micron glass fiber filter paper) into a graduated cylinder. The filter paper and beaker were washed twice with distilled, deionized water. The solution was diluted to the smallest convenient volume and transferred to an acid-washed bottle for subsequent analysis.

2. Wet Ashing

Five ml of concentrated nitric acid were added to the sample portion to be wet ashed. Six ml of concentrated sulfuric acid were then added and each beaker was covered with a watch glass. The beaker was placed on hot plate and warmed (without foaming) until the mixture became dark. The mixture was not allowed to char. The beaker was cooled. An additional sample of 5 ml of concentrated nitric acid was added to the sample and the beaker was again covered with the watch glass. The beaker was returned to the hot plate and warmed. When mixture again darkened, the beaker was cooled and 8.3 ml of 30% hydrogen peroxide were added slowly. The beaker was covered with a watch glass and heated gently until the initial reaction had ceased. If the solution became dark, the peroxide addition and heating steps were repeated several times if necessary, and heated until all fumes were driven off. If charring occurred, 1 ml portions of hydrogen peroxide were repeatedly added until the fuming sulfuric acid remained colorless or very light yellow.

Concentrated HCl (40 ml) was added and the solution was diluted to 50 ml in a graduate cylinder with deionized distilled water and transferred to an acid-washed bottle.

3. Boron and Fluoride

A portion (1-2 g) of the ground sample was weighed in nycor dishes. Five mls of saturated calcium hydroxide were added and the suspension was dried at 105°C for approximately 30 minutes. The dried material was ashed in a furnace for 1 hour at 600°C and cooled. Depending on sample size, exactly 10.0 or 15.0 ml of 0.36 N sulfuric acid were added and the material was broken up with a glass rod, gently stirred and filtered.

B. Preparation of Fish Samples

It is known that metals can be concentrated by biological processes in specific body tissues. As the fish obtained at the Crown sludge lagoon were fairly large, only a portion of the whole body would be analyzed. Therefore, upon arrival at the HAI laboratory, the fish were dissected in order to obtain portions of body tissues wherein metals would be likely to concentrate. Specifically, portions of muscle, liver, skin, scales, and bone were obtained. This dissection was conducted using plastic serrated knives so as to eliminate the possibility of metallic contamination of the sample.

The portions of bone, liver, muscle, and skin were pooled to yield a representative mix of the total metals within the fish. The sample was homogenized using a glass

mortar and pestle until a satisfactory mix was obtained. Proportionate weights of the mixture of each of the fish samples (one a duplicate) were taken and prepared by the methods described below.

1. Dry Ashing

Dry ashing (for all elements analyzed except arsenic, mercury and selenium). Approximately 10g of fish sample were weighted to the nearest 0.1g in a tarred, 100 ml tall form, acid-washed pyrex beaker. Twenty-five ml of 20 percent sulfuric acid were added. Each sample was mixed thoroughly with a glass stirring rod ensuring all sample material was wetted by the acid.

The samples were dried in an oven at 110 $\pm$ 5°C until a charred viscous sulfuric acid/sample residue was formed. Usually 12 to 16 hours were sufficient.

The samples were ashed in a muffle furnace according to the following procedure. Initially the furnace was set at 125°C and the temperature was increased approximately every hour in 50°C increments up to 275°C. The temperature was held at 275°C for 3 hours. Finally, the temperature was increased to 450°C (at 50°C per hour) and held for 12 to 16 hours. The covered ashing vessels were removed from the furnace and allowed to cool to room temperature in a clean draft-free area.

After initial overnight ashing, some residual carbon remained in some of the samples. Each sample was treated with 0.5 ml of water and 1 ml of concentrated nitric acid (whether or not they were already white). The ash was

evaporated carefully to dryness on a warm hotplate. The ashing vessels (covered with watch glasses) were placed in a cool muffle furnace and the temperature was raised to 300°C and held for 30 minutes. Each ash sample was removed from the furnace and allowed to cool as before. If residual carbon remained, the nitric acid treatment was repeated until a carbon-free white ash was obtained.

Nitric acid (0.5 ml) and 10 ml of water were added to each cool ashing vessel, then the vessel was warmed gently on a hotplate at 80 to 90°C for 5 to 10 minutes to effect dissolution of the ash. The residue was not filtered because of the possibility of contamination. The contents of each ashing vessel were quantitatively transferred into a 50 ml graduated cylinder and the volume was diluted to 30.0, 35.0 or 40.0 mls with distilled water. The solution was then transferred to an acid-washed bottle.

2. Wet Ashing

Wet ashing preparation of the fish samples was done in a manner analogous to the procedure employed for the grass samples.

3. Boron and Phosphates

The samples for boron and phosphate determinations in fish were prepared in a manner exactly analogous to the preparation of the grass samples for boron analysis.

C. Analysis of Metals in the Solutions

Quantitative inorganic metal analysis was performed on the solutions prepared as discussed previously. Most metal analysis was performed by atomic absorption on a Perkin-Elmer Model 603. Iron, zinc and manganese were present in the solutions in the parts per million (ppm) range and flame photometry was used to atmoize the sample. The rest of the elements were present in the parts per billion (ppb) range and a graphite furnace (Perkin-Elmer HGA 2100) was used to atomize the sample. Mercury was performed by the cold vapor technique. Boron was determined colorimetrically as described below.

1. Boron Determination

The sample was diluted to the range of 0.25 to 1.00 µg/ml boron. A 1.00 ml aliquot of either sample or standard was pipetted into evaporating dishes. Curcumin reagent (4.0 ml) was added to each dish and the dishes were then swirled gently to mix contents thoroughly. The dishes were floated on a water bath set at 55 $\pm$ 2°C for 80 minutes, which was sufficient for complete drying and removal of the hydrochloric acid. The drying time was kept constant for standards and samples. After the dishes cooled to room temperature, 10.0 ml of 95% ethyl alcohol was added to each dish and stirred gently with a polyethylene rod to insure complete dissolution of the red-colored product.

The contents of each dish were washed into a 25 ml volumetric flask, using 95% ethyl alcohol. The solutions were made-up to the mark with 95% ethyl alcohol and mixed tho-

roughly by inverting. The absorbance of the standards and samples was read at a wavelength of 540 nm on a Coleman II junior spectrophotometer after setting the reagent blank at zero absorbance. The calibration curve is linear from 0 to 1.00 µg/ml boron. The photometric readings were made within 1 hour of drying the samples.

If the samples were too low in boron, a known amount of boron was added to increase the concentration of boron to the range of 0.25 to 1.00 µg/ml.

2. Phosphate Determination

a. Preparation of Combined Reagent. The following reagents are mixed in this order: 50 ml 5N sulfuric acid, 5 ml 8.215 mM potassium antimonyl tartrate solution, 15 ml 0.032M ammonium molybdate solution, and 30 ml 0.1M ascorbic acid solution. The preparation was mixed after addition of each reagent. All reagents were at room temperature prior to mixing. If turbidity occurred during the combination of reagents, the mixture was shaken and allowed to stand for a few minutes until the turbidity disappeared before proceeding to the next step.

b. Treatment of the Samples. A 50 ml sample was pipetted into a clean dry test tube or 125-ml Erlenmeyer flask. One drop of phenolphthalein indicator was added. If a red color developed, 5N sulfuric acid solution was added dropwise to just discharge the color. Eight ml of combined reagent was added and mixed thoroughly. The combined reagent was less than 4 hours old. After at least 10 minutes but no longer than 30 minutes, the absorbance of each sample was measured at 880 nm, on a Coleman Junior II Spectrophotometer.

3. Fluoride

Fluoride was done by selective ion electrode using the ORION 801 pH-mV meter and the ORION F-electrode. All plastic ware was used to prevent possible glassware contamination.

D. Results

Table 5 gives the analytically determined elemental composition of the grass and fish samples collected in this study. Grass sampling sites 2, 4, 7, 9, 12, 14 and 16, and Fish B were performed in duplicate. The average values are shown to the appropriate number of significant figures in Table 5. The individual values of each duplicate are given in the quality assurance section.

E. Discussion

1. Laboratory Results

The highly variable results for iron in the Contrary Creek/D. Boyd Smith Site probably reflect the highly variable locations of the pyrite tailings. There is a slightly significant chance* that the concentration of iron in the grass from those portions of the site which had new growth was greater. If the natural logarithms of the concentrations are used the chance of significance is slightly

*Student's t = 2.03, df = 7; greater than 9 chances out of 10 but less than 95 chances out of 100 that the difference is significant, i.e., $0.1 < \alpha < 0.05$.

TABLE 5. ANALYTICAL RESULTS

Grass (μg/g) --- Contrary Creek Site	As	B	Cd	Cr	Cu	F	Fe	Hg	Li	Mn	Mo	Ni	P	Pb	Se	V	Zn
Sampling Site #1 (Control Site)	<0.2	300	<0.1	<0.1	6.7	N.R.[a]	220	<0.1	N.R.[a]	155	N.R.	1.0	50	<0.1	<0.1	N.R.[a]	60
* Sampling Site #2 (Established Growth)	<0.2	900	0.03	1.3	26	N.R.	500	<0.1	N.R.	340	N.R.	4.6	60	0.6	<0.1	N.R.	80
Sampling Site #3 (New Growth)	<0.2	1400	<0.1	<0.1	9.0	N.R.	1300	<0.1	N.R.	120	N.R.	6.6	300	4.8	<0.1	N.R.	60
* Sampling Site #4 (New Growth)	<0.2	500	0.4	1.8	17.6	N.R.	330	<0.1	N.R.	240	N.R.	2.1	345	0.8	0.6	N.R.	40
Sampling Site #5 (Established Growth)	<0.2	700	0.2	0.09	3.6	N.R.	86	<0.1	N.R.	320	N.R.	1.2	170	<0.1	0.4	N.R.	40
Sampling Site #6 (Established Growth)	<0.2	400	2.5	1.9	21	N.R.	160	0.1	N.R.	600	N.R.	4.3	140	<0.1	0.3	N.R.	1200
* Sampling Site #7 (Established Growth)	<0.2	450	0.4	0.4	7.8	N.R.	160	<0.1	N.R.	300	N.R.	3.2	100	<0.1	0.2	N.R.	70
Sampling Site #8 (New Growth)	<0.2	900	0.3	0.2	36	N.R.	180	<0.1	N.R.	410	N.R.	6.3	410	0.7	1.2	N.R.	3100
D. Boyd Smith Site																	
Sampling Site #9 (Little Growth)	<0.2	800	1.5	4.3	20.9	N.R.	1700	<0.1	N.R.	760	N.R.	8.3	180	3.5	<0.1	N.R.	400
* Sampling Site #10 (Established Growth)	<0.2	700	2.5	0.7	18.8	N.R.	70	<0.1	N.R.	470	N.R.	2.1	240	<0.1	1.2	N.R.	360
Anvil Points Site																	
Sampling Site #11	<0.2	2200	0.07	<0.1	1.4	<0.1	70.	<0.1	<0.05	18.	1.3	4.6	N.R.	<0.1	1.1	0.4	404
* Sampling Site #12	<0.2	2400	0.1	0.2	0.9	<0.1	76.	<0.1	<0.05	18.	2.4	3.8	N.R.	<0.1	3.2	<0.2	289
Sampling Site #13	<0.2	3100	0.04	<0.1	0.8	<0.1	57.	<0.1	<0.05	16.	2.8	2.2	N.R.	<0.1	4.5	<0.2	49
* Sampling Site #14	<0.2	2200	0.02	0.1	0.4	<0.1	72.	<0.1	1.0	21.	6.4	1.9	N.R.	<0.1	3.8	<0.3	22
Sampling Site #15	<0.2	1700	0.04	0.1	<0.1	<0.1	114.	<0.1	1.5	16.	2.6	0.2	N.R.	<0.1	1.0	<0.2	4.3
* Sampling Site #16	<0.2	2800	0.05	<0.1	0.2	<0.1	108.	<0.1	<0.05	28.	8.4	2.0	N.R.	<0.1	2.0	<0.2	7.8
Sampling Site #17	<0.2	1700	0.12	0.2	4.3	<0.1	57.	0.1	<0.05	18.	0.7	3.1	N.R.	<0.1	0.5	0.2	0.0
Fish (μg/g) --- Crown Site																	
Fish A	<0.2	N.R.[a]	0.1	0.1	1.0	N.R.	-- [b]	<0.1	N.R.	4.3	N.R.	2.9	N.R.	<0.1	<0.1	N.R.	3.7
* Fish B	<0.2	N.R.	0.2	0.2	0.6	N.R.	7.6	<0.1	N.R.	11.	N.R.	2.4	N.R.	<0.1	<0.1	N.R.	15.6

a. N.R. = Not required in the particular analysis.
* These samples are averages of duplicates. The actual values of each member of each duplicate is given subsequently in the Quality Assurance Section.
b This sample was lost.

greater.* Since the new growth is indicative of previous growth failure, the increased iron concentration may indicate an increased soil iron concentration which may result in growth failure due either to a toxic level of iron, sulfur, sulfuric acid produced by sulfur oxidation, or the soil pH.

The determined value for iron in the fish sample seems quite low. Correlated with this observation is the low ferric iron content observed in the settling pond waters by an independent laboratory (44). This low settling pond ferric iron content occurs in spite of the high ferric iron content identified in the raw feed (44). These data were presented in Table 3. These observations should be verified with a large number of fish samples and individual determinations on different organ systems. The mechanism for this apparent iron deficiency should be elucidated to further the understanding of synergistic and antagonistic effects.

The Anvil Points data were grouped by sampling location (i.e., 11 and 12, 13 and 14, 15 and 16). An F-test (calculated as part of Scheffe's test) shows that the variance between the pairs is significantly different than the variance within the individual values of each pair for iron (more than 975 chances out of a thousand that the observed difference is real, i.e, $\alpha < 0.025$) and zinc ($\alpha < 0.01$). Individually, the iron content in samples 15 and 16 was significantly greater (Student's t-test, $\alpha < 0.05$) than the other two sample groups and the zinc in samples 11 and 12 was significantly greater than in the other two pairs based on a Student's t-test ($\alpha < 0.05$).

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*$t = 2.26$, $0.1 < \alpha < 0.05$.

2. Quality Assurance

In order to determine the relative validity of the results, Hittman Associates, Inc. (HAI) routinely performs quality assurance analysis on all HAI products and services. In the case of analytical results, Hittman Associates traditionally evaluates the accuracy and precision of the analytical data. In this context, accuracy is defined as a measure of the nearness of the analytically derived value to the actual value of the parameter and precision is defined as a measure of the analytical reproducibility of the value. For example, if duplicate determination of a sample containing 50 µg/g iron were 25 and 75 µg/g, the accuracy of the determination would be high while the precision would be low. On the other hand, if the duplicate determinations of this same sample were 75 and 77 µg/g, the precision would be high while the accuracy would be low. Obviously, accuracy is to be prefered over precision; however, precision may serve as a measure of accuracy. Hittman Associates analytical laboratory strives to maintain high standards of both accuracy and precision.

In the absence of an absolute reference for samples collected from the environment, accuracy and precision must be estimated from data collected from the actual samples. This is most economically performed by using spiked samples (for accuracy) and by determining the values of some samples more than once (for precision). In all the samples analyzed in this report, at least two aliquots of the final solutions were determined by the atomic absorption (AA) spectrophotometer. In addition, duplicate extractions were performed on seven grass and one fish sample for a total of eight extractions or 16 AA runs per element. Four determinations were performed each on a spiked and unspiked grass pooled sample

from the Anvil Points site for a total of eight extractions or sixteen AA runs per element. The results of the duplicate AA analysis showed the precision of the machine was acceptable and did not limit the precision of the analysis significantly when compared to the limitation of precision introduced by the extraction technique. These measures of accuracy and precision are discussed below.

Table 6 shows the results of spiking grass samples. Equal aliquots of grass samples 11, 13, 17 and one of the duplicate samples of location 14 from Anvil Points were pooled. These samples were chosen in order to get an average background level. After homogenizing, the pool was divided into two sections. One section was spiked with a solution containing nickel, manganese, chromium, iron and zinc which a previous experiment indicated would give a final concentration approximately 75 percent greater than the concentration expected for that element in the pool. Previous results had indicated that lithium, cadmium, vanadium, copper and lead would be present in the pooled grass sample in such minute amounts that any attempt to spike the pool with measureable amounts of these elements would mask any interference; hence, spiking with these elements was not appropriate.

In this table, the first three columns are statistical treatment of the average values found for the individual samples which makeup the pool. The numbers in the next three columns are derived from analysis of four individual extracts of the unspiked pool. The numbers in the next three columns are derived from the spiked pool. The last column represents the percent recovery of the spike.

TABLE 6. RECOVERY EXPERIMENT ON GRASS

Element	Individual Sample Points (μg/g)			Data from Unspiked Pooled Sample (μg/g)			Data from Spiked Pooled Sample (μg/g)			% Recovery
	Arithmetic Mean	Std.Dev. (S)	Range	Arith. Mean	Std. Dev. (S)	Range	Arith. Mean	Std.Div. (S)	Range	
Nickel	2.8	1.3	1.5-4.6	2.7	0.7	1.8-4.0	4.2	0.5	3.6-4.9	71.
Manganese	18.	2.	16.-21.	17.	0.5	16.-17.	29.	2.	27.-33.	76.
Chromium	0.14	0.05	<0.1-0.2	0.12	0.09	0.056-0.304	0.27	0.07	0.12-0.36	100.
Iron	62.	6.	57.-70.	88.	5.	80.-93.	151.	19.	127.-177.	105.
Zinc	120.	190.	0.0-404.	54.	2.	52.-56.	126.	2.	123.-128.	90.

The data from the spiked grass samples shows generally good recovery. The average of the pool determinations is never significantly different (at the 95 percent level) than the average of the individual samples. The average recovery for all spiked elements is approximately 90 percent which indicates the extraction procedure is remarkably efficient. The low standard deviation of the pooled samples relative to the individual determination shows that the precision of the method is quite sufficient to determine differences between samples taken from different locations within the same general site.

A similar study was performed using a spiked and unspiked sample of fish muscle. Muscle was used since all of viscera and bone was being reserved for analysis. The percent recovery of some of the metals present in the tissue is given below:

Metal	Percent Recovery
Nickel	118
Cadmium	78
Zinc	102
Manganese	80
Chromium	127

The results are not as definitive as the grass samples. The recovery experiments indicate that the procedure as applied is accurate with respect to the nickel, cadmium, zinc and magnesium in fish tissue. The results with chromium are ambiguous. The recovery experiments with lead and copper indicated that the determinations of these metals in fish tissue were questionable and possibly below Hittman Accuracy Standards and that these metals should be redetermined with possible variations in methodology. The analysis of duplicate determinations of unspiked fish samples (Table 7) also shows questionable precision of the analysis of zinc and

TABLE 7. RESULTS OF DUPLICATE DETERMINATIONS (µg/g)

Contrary Creek/ D. Boyd Smith	As	B	Cd	Cr	Cu	F	Fe	Hg	Li	Mn	Mo	Ni	P	Pb	Se	V	Zn
Sampling Site #2	<0.2	1180.	0.00	0.2	16.	N.R.[a]	285.	<0.1	N.R.	346.	N.R.	5.9	50.	1.1	<0.1	N.R.	123.
	<0.2	680.	0.06	2.4	35.	N.R.	714.	<0.1	N.R.	329.	N.R.	3.3	70.	<0.1	<0.1	N.R.	43.
Sampling Site #4	<0.2	420.	0.4	1.0	23.	N.R.	404.	<0.1	N.R.	250.	N.R.	1.5	360.	1.1	0.6	N.R.	50.
	<0.2	620.	0.4	2.5	12.	N.R.	262.	<0.1	N.R.	231.	N.R.	2.7	330.	0.5	0.5	N.R.	23.
Sampling Site #7	<0.2	290.	0.4	0.4	8.9	N.R.	167.	<0.1	N.R.	288.	N.R.	0.7	100.	<0.1	0.3	N.R.	100.
	<0.2	610.	0.3	0.5	6.7	N.R.	159.	<0.1	N.R.	308.	N.R.	5.7	110.	<0.1	0.2	N.R.	35.
Sampling Site #10	<0.2	650	3.0	0.5	22.	N.R.	93.	<0.1	N.R.	504.	N.R.	2.5	240	<0.1	1.6	N.R.	448.
	<0.2	710.	2.0	0.9	15.	N.R.	47.	<0.1	N.R.	430.	N.R.	1.7	230	<0.1	0.7	N.R.	273.
Anvil Points																	
Sampling Site #12	<0.2	2600.	0.00	0.4	0.8	<0.1	78.	<0.1	<0.05	17.	3.1	3.3	2600.	<0.1	3.4	<0.2	289.
	<0.2	2100.	0.14	<0.1	1.0	<0.1	74.	<0.1	<0.05	18.	1.8	4.4	2100.	<0.1	3.0	<0.2	S.L.[b]
Sampling Site #14	<0.2	2300.	0.03	0.1	0.6	<0.1	79.	<0.1	1.9	21.	7.4	2.3	2300.	<0.1	2.7	<0.3	26.
	<0.2	2000.	0.01	<0.1	0.3	<0.1	65.	<0.1	<0.05	21.	5.5	1.5	2000.	<0.1	4.8	<0.2	18.
Sampling Site #16	<0.2	3300.	0.06	<0.1	0.2	<0.1	94.	<0.1	<0.05	18.	8.1	0.6	3300.	<0.1	1.3	<0.2	6.0
	<0.2	2200.	0.04	<0.1	0.1	<0.1	122.	<0.1	0.05	37.	8.8	3.4	2200.	<0.1	2.6	<0.2	9.5
Crown																	
Fish B	<0.2	N.R.	0.1	0.1	0.8	N.R.	8.7	<0.1	N.R.	1.2	N.R.	3.2	N.R.	<0.1	<0.1	N.R.	9.9
	<0.2	N.R.	0.3	0.3	0.5	N.R.	6.5	<0.1	N.R.	20.4	N.R.	1.6	N.R.	<0.1	<0.1	N.R.	21.3

[a] N.R. = This value was not required for the analysis.

[b] S.L. = Sample lost.

manganese.

The inaccuracy of the copper and lead determinations in fish tissue and the imprecision in the determinations of manganese and zinc in fish tissue indicate that these metals should be redetermined in fish tissue. Unfortunately, comparable samples of fish tissue were unavailable so that further analysis of these samples, although desirable, is not possible.

Table 8 shows the results of Scheffe's Test on the duplicate determinations of some of the samples. Duplicate determinations were not performed on each sample since this would lead to prohibitively high cost. Scheffe's F-test shows that the difference between all values is significantly higher (at the 99.9% level) than the difference between the duplicates. This is not surprising since there is a large variability in the data. (For example, arsenic is always less than 0.2 µg/g while the average of one iron sample is 500 µg/g). So the F-test was applied to each individual element. The results presented in Table 8 indicate that the variability between the determinations is greater than the variability between the duplicates for cadmium, copper, selenium, iron, manganese, molybdenum, phosphorus and zinc.

The inability to demonstrate a difference between the points greater than the difference between duplicates of the same point is not surprising in the case of arsenic, mercury, lithium, vanadium, and fluoride since all these values were below detection limits.

The lack of precision in the concentration of zinc and manganese in fish tissue is disturbing and may result from

TABLE 8. SCHEFFE'S TEST ON DUPLICATE DETERMINATIONS FOR EACH ELEMENT

Element	Scheffe's Test	Probability of a Type I Error*
Boron	$F(6,7) = 13.68$	$\alpha < 0.01$
Cadmium	$F(7,8) = 21.04$	$\alpha < 0.01$
Chromium	$F(7,8) = 1.63$	$\alpha > 0.05$
Copper	$F(7,8) = 6.16$	$\alpha < 0.025$
Iron	$F(7,8) = 4.27$	$\alpha < 0.05$
Lead	$F(7,8) = 1.88$	$\alpha > 0.05$
Lithium	$F(2,3) = 1.00$	$\alpha = 1$
Manganese	$F(7,8) = 143.8$	$\alpha <<< 0.01$
Molybdenum	$F(2,3) = 19.34$	$\alpha < 0.025$
Nickel	$F(7,8) = 0.70$	$\alpha > 0.05$
Phosphorus	$F(6,7) = 26.36$	$\alpha << 0.01$
Selenium	$F(7,8) = 9.38$	$\alpha < 0.01$
Zinc	$F(6,7) = 10.32$	$\alpha < 0.01$

*A type I error is the chance of indicating that something is significant when it actually is not. An $\alpha < 0.01$ indicates that there is less than one chance in 100 that the observed relationship is due solely to chance. The F-test cannot be applied to arsenic, fluoride, mercury and vanadium in this case.

the lack of homogenity of the sample of liver, bone, skin and muscle (i.e., one of the duplicates may have had most of the bone tissue). A much more interesting study would have been to collect enough fish of the same species to get statistically significant fractions of each tissue from many fish from the pond and in a neighboring uncontaminated (control) waterbody. A sample of two fish does not lend itself to statistical treatment.

As another check, the significance of the difference between the individual elements at the Contrary Creek/D. Boyd Smith location and the Anvil Points location was determined using Student's t-test (with 15 degrees of freedom). Logically, some differences should be apparent between these two data sets.

From Table 9 it can be seen that the values for boron, copper, manganese and selenium differ significantly between the two sites. Since the analyses were done under identical onditions, the analytical procedure appears to be able to identify the site from which the sample was gathered.

3. Recommendations

a. A more extensive study which relates the concentration of various metals in the soil to those in the grass needs to be undertaken. This study should also involve analysis of some elements in other animals such as rodents, groundhogs, foxes, etc., which may reasonably be expected to obtain their nourishment from the site (as confirmed by stomach contents of the herbivorous animals either line-trapped or within the stomach of the carnivore. Bioconcentration factors (soil to grass to herbivorous animal to

TABLE 9. STUDENT'S T-TEST TO DETECT THE DIFFERENCE BETWEEN ANVIL POINTS AND CONTRARY CREEK/D. BOYD SMITH

Element	Student's	Probability of a Type I Error*
Boron	$t = 7.82$	$\alpha < 0.001$
Chromium	$t = 1.96$	$\alpha < 0.2$
Copper	$t = 4.03$	$\alpha < 0.01$
Iron	$t = 1.82$	$\alpha < 0.2$
Lead	$t = 1.56$	$\alpha < 0.2$
Manganese	$t = 4.67$	$\alpha < 0.001$
Nickel	$t = 1.36$	$\alpha < 0.2$
Selenium	$t = 3.36$	$\alpha < 0.01$
Zinc	$t = 1.15$	$\alpha < 0.2$

*A type I error is the chance of indicating that something is significant when it actually is not. An $\alpha < 0.01$ indicates that there is less than one chance in 100 that the observed relationship is due solely to chance. A 1.0 indicates that there is no chance of the data being different.

carnivorous animal) could then be computed. The animals could also be examined for any pathological abnormalities which could be related to the site. The second part of this study should include vegetation and animals from a neighboring but uncontaminated similar site. These data will be used as a control for comparison with those from potentially contaminated area.

Earthworms can also be used as an indicator species to indicate environmental health conditions. The analysis of grass should be conducted on a tissue-specific basis (leaves vs. stems vs. roots vs. flowers, etc.).

b. A more extensive study of a large sample of fish, or other organisms (particulary benthic), is recommended. Likewise, separate analysis of organ systems and fish from a nearby uncontaminated source is needed to serve for comparison. The low iron values obtained in this study should be verified. If they are found to be true, the mechanism of the apparent iron imbalance should be eluciated. This study should also attempt to correlate the concentration of elements in the fish with those from pond water.

c. Procedures a. and b. above should be performed periodically, preferably every season for a number of years, in order to observe trends in bioconcentration and bioaccumulation of metals and other elements associated with mining wastes. The EPA analyses to date should be included in the analysis.

REFERENCES

1. Plant Performance on Surface Coal Mine Spoil in Eastern United States, SCS-TP-155, Soil Conservation Service, U.S. Department of Agriculture, Washington, D.C., April, 1978.

2. Ross, R.H. "Environmental Interactions." In: Environmental, Health, and Control Aspects of Coal Conversion: An Information Overview. Vol. 2, ed. by H.M. Braunstein, E.D. Copenhaver, and H.A. Pfuder. Oak Ridge Information Center Complex, Oak Ridge National Laboratory, 1977.

3. Bowen, H.J.M. Trace Elements in Biochemistry. New York: Academic Press, 1966.

4. Lisk, D.J. "Trace Metal in Soils, Plants, and Animals." In: Adv. in Agronomy Vol. 24, ed. by N.C. Brady, New York: Academic Press, 1972, pp. 267-325.

5. Dvorak, A.J. et. al., The Environmental Effects of Using Coal for Generating Electricity. NUREG-0252, Available from NTIS, Department of Commerce under No. PB267-237, 1977.

6. Fowler, S.W., and G. Benayoun. "Experimental Studies on Cadmium Flux Through Marine Biota." Comparative Studies of Food and Environmental Contamination, Vienna: International Atomic Energy Agency, 1974.

7. Unpublished information submitted to the U.S. Environmental Protection Agency under Contract No. 77-43-302073.

8. Parris, G.E. and F.E. Brinckman. "Reactions which relate to environmental mobility of arsenic and antimony II: Oxidation of trimethyarsine and trimethylstibine." Environmental Science and Technology. Vol. 10, No. 12 (1976).

9. Environmental Protection Agency. Arsenic. Washington: National Academy of Sciences, 1976.

10. Fishbein, L. Second Task Force on Research Planning in Environmental Health Science Background Document Metals and Metalloids: Mercury, Lead, Cadmium, Arsenic and Selenium.

11. Woolson, E.A. "Organoarsenical Herbicides," In: Arsenical Pesticides. Washington: American Chemical Society, pp. 741-776.

12. Woolson, E.A. "Bioaccumulation of Arsenicals." In Arsenical Pesticides. Washington: American Chemical Society. pp. 97-107.

13. Schulz-Baldes, M. "Lead uptake from sea water and food, and lead loss in the common mussel Mytilus edulis." Marine Biology. Vol. 25(1974) pp. 177-193.

14. Wong, P.T.S., Y.K. Chau, and P.L. Luxon, "Methylation of lead in the Environment." Nature. Vol. 253, No. 1, pp. 263-264.

15. Dailey, N.S. "Process Effluents: Quality and Control Technologies." In Environmental, Health and Control Aspects of Coal Conversion: An Information Overview. ed. by H.M. Braustein, E.D. Copenhaver and H.A. Pfuderer. Oak Ridge: Information Center Complex, Oak Ridge National Laboratory, 1977.

16. Gehrs, C.W. Lifescience program Synthetic fuels.

17. Henkin, R.I., J.R. Marshall, and S. Meret. "Maternal-fetal metabolism of copper and zinc at term." American Journal of Obstetrics and Gynecology, Vol. 110, No. 1, (1971), pp. 131-134.

18. Silverberg, B.A., P.T.S. Wong, and Y.K. Chau. "Ultra-structure Examination of Aeromonas cultured in the presence of organic lead." Applied and Environmental Microbiology. Vol. 11 (1976) pp. 723-725.

19. Andelman, J.B. and M.J. Suess. "The Photodecomposition of 3,4-Benzpyrene Sorbed on Calcium Carbonate. In: Organic Compounds in the Aquatic Environment. ed. by S.D. Faust and J.V. Hunter. New York: Marcel Dekker Inc. 1971.

20. Mason, J.W., J.H. Cho, and A.C. Anderson. "Uptake and loss of inorganic mercury in the eastern oyster (Crassostrea virginica)." Archives of Environmental contamination. Vol. 4, (1976), pp. 361-376.

21. Wilkes, D.J. "Animals: Bioenvironmental Effects." In: Environmental, Health, and Control Aspects of Coal Conversion: An Information Overview Vol. 2. Oak Ridge: Information Center Complex, Oak Ridge National Laboratory, 1977.

22. Vaugham, B.E. et. al., Review of Potential Impact on Health and Environmental Quality from Metals Entering the Environment as a Result of Coal Utilization. A Battelle Energy Program Report, Pacific Northwest Laboratories, Battelle Memorial Institute, Richland, Washington 99352, 1975.

23. Po-Yung Lu. et al. "Model ecosystem studies of lead, and cadmium and of urban sewage sludge containing these elements." J. Env. Qual. Vol. 4, No. 4 (1975), pp. 505-509.

24. Paschal, D.C., and W. McNamara. "Uptake of cadmium and nickel by salvania." Vol. 68, No. 2 (1975) pp. 132-135.

25. Greig, R.A., D.R. Wenzloff, and J.B. Pearce. "Distribution and abundance of heavy metals in finfish, invertebrates and sediments collected at a deepwater disposal site." Marine Pollution Bulletin, Vol. 7, No. 10 (1976) pp. 185-187.

26. Carrier, R.F. "Plant Interactions" In: Environmental, Health, and Control Aspects of Coal Conversion: An Information Overview. Vol. 2. Oak Ridge: Information Center Complex, Oak Ridge National Laboratory, 1977.

27. Gordon, C.C. Effects of Air Pollution on Indigenous Animals and Vegetation. In: Helena Valley Environmental Pollution Study. pp. 95-112.

28. Van Hook, R.I., "Cadmium, lead, and zinc distributions between earthworms and soils; Potential for biological accumulation." Bulletin of Environmental Contamination and Toxicology, Vol. 12, No. 4 (1974), pp. 509-512.

29. Ireland, M.P. "The effect of the earthworm, Dendrobaena rubida on the solubility of lead, zinc, and calcium in heavy metal contaminated soil in Wales." Journal of Soil Science, Vol. 26, No. 3 (1975), pp. 313-318.

30. Subramanian, V. and B. D'Anglejean. "Wate chemistry of the St. Lawrence Estuary." Journal of Hydrology. Vol. 29 (1976), pp. 341-354.

31. Dietz F. "The Enrichment of Heavy Metals of Submerged Plants." In: Advances in Water Pollution Research. 6th International Conference in Jerusalem, 1972, ed. S.H. Jenkins. New York: Pergamon Press, 1973, pp. 53-63.

32. Emerson, R., et. al. "Heavy metal concentrations in marine organisms collected near an industrial waste outfall." Institute of Electrical and Electronics Engineers, Inc., 1976, pp. 1-5.

33. Gish, C.D. and R.E. Christensen. "Cadmium, nickel, and zinc in earthworms from roadside soils." Environmental Science and Technology, Vol. 7, No. 11 (1973), pp. 1060-1062.

34. Penden, J.D., J.H. Crothers, C.E. Waterfall, and J. Beasley. "Heavy metals in Somerset marine organisms." Mar. Poll. Bull., Vol. 4 (1973), pp. 7-9.

35. Krauskopf, K. "Factors controlling the concentration of thirteen rare metals in seawater." Geochim. Cosmochim. Acta, Vol. 9 (1956), pp. 1-32B.

36. Martin, M.H. and P.J. Coughtrey. "Comparison between the levels of lead, zinc, and cadmium within a contaminated environment." Chemosphere, Vol. 1 (1976), pp. 15-20.

37. Saward, D., A. Stirling, and G. Topping. "Experimental studies on the effects of copper on a marine food chain." Marine Biology, Vol. 29 (1975), pp. 351-361.

38. Baptist, J.P., and C.W. Lewis. "Transfer of 65ZN and 51Cr through an estuarine food chain." In Symposium on Radioecology, Ann Arbor, 1967. ed. D.J. Nelson and F.C. Evans, U.S. Department of Commerce, CONF-670503 1969, pp. 420-430.

39. Huckabee, J.W., and B.G. Blaylock. "Transfer of Mercury and Cadmium from Terrestrial to Aquatic Ecosystems." In Metal Ions in Biological Systems, ed. S.K. Dhar. Plenum Publishing Corporation, pp. 125-159.

40. Bastian, R.K. and W.B. Jackson. "137Cs and 60Co in a terrestrial community at Enewetak Atoll." Radioecology and Energy Resources: Proceedings of the Fourth National Symposium on Radioecology, Oregon State University, Corvalis, Oregon, May 12-14, 1975. ed. by C.E. Cushing et al. Stroudsburg, PA: Dowen, Hutchinson and Ross, Inc., 1976.

41. Mathis, B.J. and T.F. Cummings. "Selected metals in sediments, water, and biota in the Illinois River." Journal of the Water Pollution Control Federation. Vol. 7, pp. 1573.

42. McDermott, D.J., G.V. Alexander, D.R. Young, and A.J. Mearns. "Metal contamination of flatfish around a large submarine outfall." Journal of Water Pollution Control Federation, Vol. 48, No. 8 (1976), pp. 1913-1918.

43. Environmental Protection Agency. Preliminary Investigation of Effects on the Environment of Boron, Indium, Nickel, Selenium, Tin, Vanadium, and their compounds. Volume III Nickel. Office of Toxic Substances. EPA-560/2-75-005C (1975), pp. 1-89.

44. Information obtained from Mr. Jim Kennedy, Project Officer, EPA Crown Coal Mine Wastewater Treatment Facility, Riversville, West Virginia.

45. Information obtained from Mr. Ken Hinkle, Virginia State Water Quality Control Board, Bridgewater, Virginia.

46. Mr. Ed Bates and Mr. Terry Thome, U.S. Environmental Protection Agency, Cincinnati, Ohio.

47. J.R. Nelton, W.L. Hoover, P.A. Howard and J.L. Ayers, J. Assoc. Offic. Anal. Chem. 53, 682 (1970).

48. Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue, U.S. Environmental Protection Agency, Environmental Monitoring and Support Lab., Cincinnati, Ohio.

49. Standard Methods for the Examination of Water and Wastewater, Fourteenth Edition, Copyright 1976.

50. ORION, Instruction Manual for Fluoride Electrodes Copyright 1976, Orion Research, Inc.

51. Methods of Analysis of the Association of Official Analytical Chemists, 12th Edition, 1975, Published by the Association of Official Analytical Chemists, Washington, D.C.